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THE EFFECTS OF TEMPERATURE AND MOISTURE UPON THE BEHAVIOUR OF THE SPRUCE BUDWORM, *CHORISTONEURA FUMIFERANA* CLEMENS (LEPIDOPTERA: TORTRICIDAE)

I. THE RELATIVE IMPORTANCE OF GRADED TEMPERATURES AND RATES OF EVAPORATION IN PRODUCING AGGREGATIONS OF LARVAE¹

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INTRODUCTION

This paper is the first of a series dealing with the effects of temperature and rate of evaporation upon the behaviour and activity of the spruce budworm, *Choristoneura fumiferana* (Clemens). Much of the work was concerned with the orientation reactions exhibited by the six larval stages in response to stimulation by these variables. The reactions studied most thoroughly were those which resulted in the aggregation of groups of larvae in what are commonly termed "preferred" zones. Although the studies reported in this and a succeeding paper were carried on simultaneously and are, in some respects, not easily separable, it is necessary, for the present, to distinguish between behaviour in gradients of the combined variables and behaviour in gradients of the single variable, evaporation. Thus, in the following pages, the descriptions of methods and of apparatus will in large part apply to work discussed in the subsequent paper also.

Although the work reported in this series is part of an investigation designed to provide data for the development of a number of different types of studies of the effects of weather and climate upon the spruce budworm, in the present paper the animal has been used to demonstrate an academic point.

Rate of evaporation, despite objections stemming from the lack of easily comparable units, was used in the present work to demonstrate a relationship which would have been masked by measurements of relative humidity or saturation deficit. This is dealt with at greater length in the discussion, but it is beyond the scope of this paper to extend the controversy on the relative merits of the various methods of expressing atmospheric moisture for biological purposes. Those who may prefer the earlier arguments of Mellanby (8) and Anderson (1) are referred to the papers of Ramsay (9), Leighly (7) and Thornthwaite (13).

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EXPERIMENTAL MATERIAL

Unless otherwise noted, the insects used in the experiments were collected near Lake Nipigon, Ontario, during October, 1945. Branches of balsam fir infested with overwintering larvae were stored first under snow cover in a screened insectary, and, later, before the spring thaw in March, 1946, were transferred to a cold room held between 2.5 and 4.5° C. Bundles of the foliage were brought from cold storage as required, and emerging larvae were collected with a brush or a pump-driven aspirator and reared at 20-21° C. until reaching the required instars.

APPARATUS

Two types of gradients were used in the experiments. The design of the apparatus to produce the gradients used presented no particular problem, but it was necessary to find some means of measuring the rates of evaporation within them. This instrument will be treated first.

The Evaporimeter

This instrument had to be small enough to be enclosed in the confines of a gradient, yet capable of measuring the rates of evaporation within a gradient rapidly enough so that the slope of the gradient of evaporation could be changed quickly, if desired. Ordinary spherical atmometers were too large for the size of the apparatus used. However, Ramsay, Butler and Sang (10) described a capillary-tube evaporimeter which was used as the basis for the model fashioned for use in this investigation.

In its original form, the instrument of Ramsay *et al.* was roughly comparable to the Piche evaporimeter. However, it was constructed on a much smaller scale. It consisted of a disc of cigarette paper 2 mm. in diameter, which was attached to one end of a capillary tube filled with water. The rate of evaporation was determined by observing the movement of the meniscus along the bore of the tubing. This was done with the aid of a travelling microscope used with the unit.

For the purposes of this investigation, the microscope was discarded, since such an arrangement was too bulky. White-backed thermometer tubing of 0.5 mm. bore was substituted for ordinary capillary tubing. The white tubing gave enough contrast so that the position of the meniscus could be determined from ordinary reading distance. Lens-fronted, red-backed thermometer tubing might have been employed to give an even greater contrast, but it was not available at the time when the instruments were required. Its use is recommended for future patterns.

The millimeter scale was drawn in India ink on the gummed side of a strip of transparent cellulose tape, which was then fixed in position along the bore of the tubing. This type of scale proved to be extremely durable if it was painted over with a thinned solution of cellulose acetate. Filter paper discs 4 mm. in diameter were used in place of cigarette paper, since the only available brand of the latter was not suitable for an evaporative surface. The filter paper used was a loose-textured type (Cenco No. 13255).

The net result was an instrument of pocket-size (Figure 1) which could be placed in spaces of very restricted volumes. Since it lacked an attached microscope, it could not be read instantaneously, but this proved to be

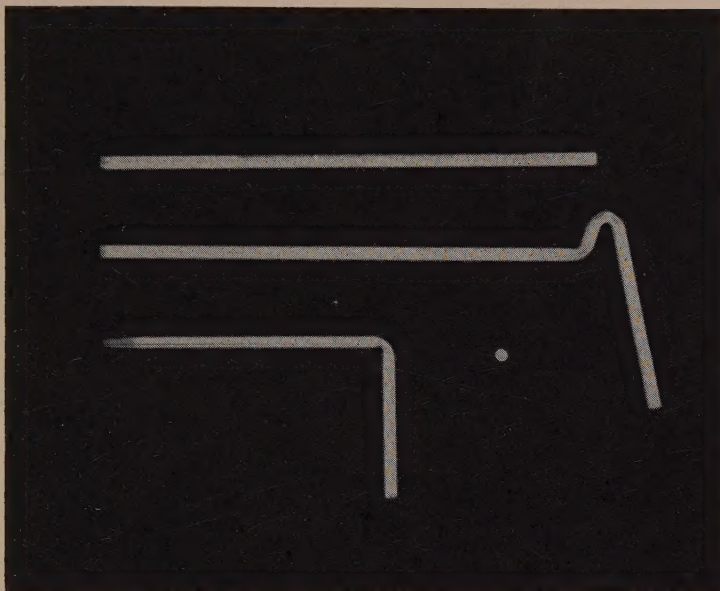


FIGURE 1. Sample types of capillary-tube evaporimeters fashioned from white-backed thermometer tubing. The straight tube at the top was used for measurements reported in this paper. The filter paper disc appears at the lower right of the illustration (disc diameter, 4 mm.).

unimportant during the measurements, for stable conditions commonly existed. The times required for reading the rates of evaporation varied from 2.5 minutes for rapid rates to 10 minutes for very low rates close to actual saturation. Over most of the range, 5 minutes was the interval employed. Since stable conditions existed at any point, readings were converted to cu. mm. per minute.

The Evaporation Gradient Apparatus

For observations on the reactions of larvae to evaporation at a constant temperature, apparatus producing a linear, rather than a concentric gradient was constructed, since available materials did not lend themselves to the construction of the latter type. This apparatus was much the same as that described by Sokolov (11). It consisted of a floorless box of black "Masonite" 45 cm. long, 8 cm. wide and 2.8 cm. high. A glass top was cut to fit and could be sealed with cellulose tape. Another box was constructed to fit snugly within the original box. The second frame was 5 mm. lower than the first, so that, when topped by a platform, it formed the floor of the experimental chamber. Fifty-one mesh nylon was used for the experimental platform. When it was placed loosely over the top of the second box, forcing the outside box down over the whole drew the nylon taut. A line of Syracuse dishes was placed beneath the experimental platform. The dishes contained, in sequence, wet cotton, wet and dry sodium carbonate and wet and dry calcium chloride. Usually only about 35 cm. of the length of the gradient was used, except in certain experiments with light, so that, as a rule, all the dishes were placed within this area. The rest of the gradient was then blocked off so that larvae were confined to the working area.

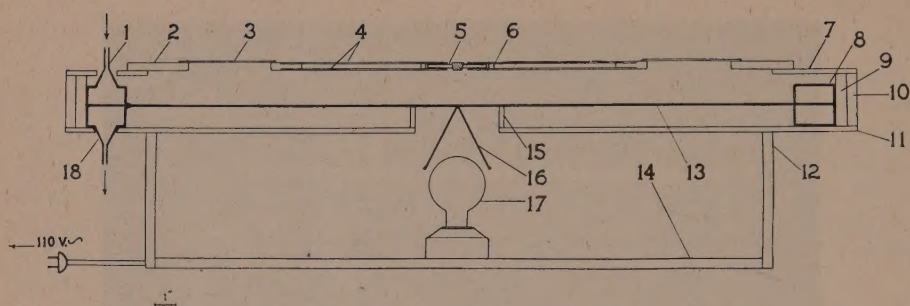


FIGURE 2. Apparatus for producing gradients of temperature and evaporation: Sectional elevation along one diameter. 1, water inlet; 2, roof; 3, port covers; 4, transparent double roof; 5, slide; 6, bracing strip; 7, supporting frame; 8, marginal cooling duct; 9, bracing strip; 10, supporting frame; 11, supporting frame; 12, base; 13, brass disc forming working surface of gradient; 14, mounting board for lamp; 15, bracing strip; 16, heat-conducting cone; 17, heating lamp; 18, water outlet.

The Combined Temperature and Evaporation Gradient Apparatus

Most of the literature describing the various types of apparatus devised to study the temperature preferences of terrestrial animals, with or without provision for the study of the relation of atmospheric moisture to these aggregations, has been summarized by Herter (6), Fraenkel and Gunn (4) and Deal (3), so that it need not be included here. For this investigation, it was decided that a concentric gradient (cf. Totze (14)) would best meet the particular needs. The apparatus devised had some special features which are described below (see also Figure 2).

The working surface of the gradient consisted of a brass disc 3 mm. thick and 90 cm. in diameter. To the centre of the disc, on its lower surface, was soldered a truncated copper cone 7 cm. deep and 7 cm. in basal diameter. This served to conduct heat from the source, a 40-watt lamp, to the centre of the disc, whence it passed to the periphery.

Around the margin of the disc was drilled a row of 1 cm. holes, spaced at 15 cm. intervals. Enclosing these there was a water-tight copper duct, which was soldered to the adjacent surfaces of the disc around its entire margin. This duct was 5 cm. square and 90 cm. in outside diameter. As the duct served as a marginal cooling ring, a water inlet and an outlet were provided, the former on the upper surface of the duct and the latter on the lower surface, 5 cm. along the duct from the position of the inlet. The inner passage immediately between the two was sealed by means of two 2.5×5 cm. strips of copper soldered to the inner surfaces of the duct and the enclosed disc, so that the introduced coolant was forced around the entire periphery between the times of its entrance and exit.

The metal work was set in a shallow, square plywood box just deep enough to hold it. The box, together with a square wooden base, served as both support and insulation. The lamp which served as a heat source was mounted on a detachable board on the base to permit replacement of the lamp. In the lid of the box was cut a circle 75 cm. in diameter. Over the hole was placed a detachable roof, consisting of a plywood disc 80 cm. in diameter. On one diameter, two 10×12.5 cm. ports were cut through

the roof, each placed 7.5 cm. from the adjacent margin of the disc. The central portion of the roof consisted of two sheets of 3 mm. cellulose acetate separated by a 6 mm. air-space to form a double roof which provided an observational area measuring 37.5×75 cm. at its widest point. Two bracing strips separated the sheets near the centre line of the roof. Between the braces, and partially enclosed by the acetate sheets, lay a "Lucite" slide, $0.6 \times 6 \times 80$ cm. Two 2.5×37.5 cm. slots were cut through the sheets along the radius above and below this slide, to facilitate introduction of thermocouples or material through two holes drilled near the centre of the slide.

To set the apparatus in operation, the roof was set in place, glass slides were placed over the ports, and the light and the water supply (see below) were turned on. The apparatus was always used in a rearing room held between 20° and 21° C. Under these conditions, a working gradient could be established in 15 minutes, and could be maintained indefinitely.

There was available a water-storage tank which was held at a constant low temperature by means of a "Freon" cooling coil. A line from this was connected to the inlet of the cooling duct of the apparatus. With a rate of flow of 1 litre per minute, it was found that the temperature of the incoming water was held at 7° C., and that the water warmed to 7.5° in its circuit of the duct. Thus, the isotherms on the working surface were not strictly concentric. Nevertheless, they could be considered so for practical purposes, since the half-degree difference between incoming and outgoing water diminished to a peripheral difference of 0.1° when measured on the disc.

The gradient of temperature obtained amounted to 30 Centigrade degrees ($40^{\circ} - 10^{\circ}$) over a radial distance of 40 cm. Although the fall was not regular, no undesirable effects were noted in the work. It was possible to measure the surface temperature at any point with only one searching junction, by setting the "Lucite" slide and rotating the roof until the hole in the slide came to the desired position.

The amount of moisture in the enclosed air was changed by attaching moistened cotton pads or sacks of drying agents to the under surfaces of the opaque plates covering the ports. As long as the roof was kept in one position, the moisture gradient remained concentric except in the immediate vicinity of the pads, where the regularity of the iso-lines was destroyed. The reactions of the insects kept this from becoming a problem in the observational work. A number of stable gradients of evaporation were obtained which could be checked at a few standard points.

A disadvantage of this apparatus stemmed from the fact that it was impossible to obtain suitable materials for its construction during 1945. The roof should have been completely transparent. In the present work, it so happened that accurate observations could still be made, because the behaviour of the larvae eliminated the difficulty. However, the objection still stands if similar apparatus is to be used with other species of different habits.

METHODS

The "bar" evaporation gradient was always used at room temperature, and hence was ready for use at any time. Its stability was checked with the evaporimeter before and after any experiment. The temperature gradient proper was left running day and night. One half-hour before an experiment began, the appropriate evaporation gradient was established in the apparatus. Both the temperature and evaporation gradients in the apparatus were checked at three points along a radius of the disc before an experiment and during the latter half of any one hour's observation, and the values obtained checked against the original calibration curves.

Insects were introduced into either apparatus with a brush. They were distributed evenly along or throughout the working area, with the important exception that, in the temperature gradient, larvae never were dropped above the 36° C. isotherm. It was observed that small larvae dropped into zones of temperature higher than this seemed unable to orientate quickly enough to escape before being injured by the high temperature. On the other hand, if larvae wandered into the upper zone while moving about the disc, they were able to reorientate quickly. Thus, it was thought to be better to let them wander into this zone rather than to drop them into it.

The time at which the last larva of a group was placed in any apparatus was reckoned as zero time for the observation which followed. If instars 1 to 3 were used, it was necessary to check the condition of these larvae after an interval of five minutes, because a few of them were occasionally injured by the brush and became inactive. They were removed and replaced by others of the same instars held in reserve for this purpose. The number of necessary replacements was never more than 3 per cent, and it was considered better to replace injured and sluggish larvae before the actual observations began rather than risk the occurrence of a spurious peak in a final distribution such as would be caused by these larvae remaining at one point.

The first record of the positions of the larvae was made fifteen minutes after the zero of time. The first three instars of the spruce budworm moved about almost incessantly on a bare surface, but, with practice, it was possible to record the positions of 100 larvae, the usual number in a group, in 2.5 minutes.

It was found that four observations in any one hour on 100 larvae were more than sufficient to establish the distribution peculiar to the time interval. The last three observations in any one hour were taken at 30, 45 and 60 minutes, as reckoned from the zero of time.

The positions of the larvae in both types of apparatus were always recorded in terms of distance from a fixed point on the gradients rather than in terms of the units of temperature or evaporation employed. This method of recording positions raised no problem in the narrow evaporation gradient, but in the concentric gradient, concentric rings spaced 6 mm. apart had to be shallowly enscribed in the surface of the brass to provide some means of locating the larvae. These rings were numbered from the centre to the periphery. During each observation, the number of larvae in each ring was recorded, and the results later translated into class intervals

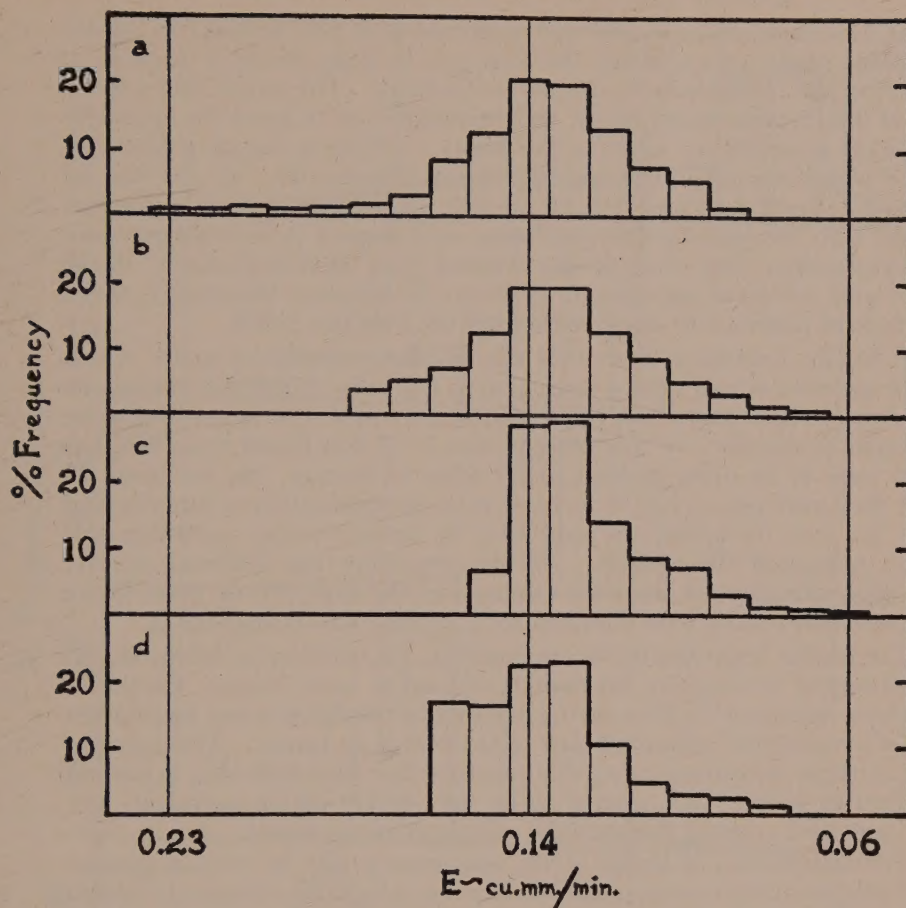


FIGURE 3. Aggregations in response to evaporation exhibited by four groups of initially moist second stage larvae in four types of gradients: *a*: in a dry temperature gradient where the 36° C. isotherm lay above an evaporative rate of 0.23 cu. mm. per min.; *b*: in a moist temperature gradient, with the 36° C. isotherm above 0.18 cu. mm. per min.; *c*: in a very moist temperature gradient, with the 36° C. isotherm above 0.15 cu. mm. per min.; *d*: in a gradient of evaporation which did not extend above 0.16 cu. mm. per min., but which was at a constant temperature of 20.6° C. Ordinate: percentage frequency; abscissa: rate of evaporation, cu. mm. per min.

of evaporation or temperature. No larvae of any instar reacted to the presence of these rings. Furthermore, no larva was ever observed to react to the silk trails of other larvae, but, as a precaution against eventual occurrence of such behaviour, the surfaces of the gradients were always wiped clean after any experiment was completed.

RESULTS

It soon became apparent that the previous treatment of the larvae under observation was of the utmost importance. If this treatment was not kept within rigid limits, inconsistencies developed among the results of observations on supposedly similar groups of larvae.

It was found that, in any one experiment, it was possible to obtain repeatable observations within the first two or three hours of the run, if the larvae had been similarly treated beforehand. Thereafter, the original type of distribution broke down, and bimodality or trimodality appeared. This state persisted for some twelve hours. At times, single peaks were formed which resembled the original type of distribution. In the last six hours of a twenty-four hour period, another stable type of distribution was formed, with the peak considerably lower with respect to both temperature and evaporation than that of the original type of distribution. If the larvae were not given any special treatment in advance, bimodality or the final type of distribution appeared during the first two hours.

It finally became evident that the moisture conditions under which the larvae were stored before testing were the most important conditions involved. (These points will be treated further in a later paper, but some indication is necessary in this presentation.) It was found that, whether larvae were to be dried or kept moist while in storage, the container in which they were placed had to be lined with the desiccating or humidifying agent, so that there was no possibility of larvae resting anywhere but on the surface of the reagent. If this procedure was followed rigidly, bimodality disappeared from the readings of the first two or three hours and consistent results were obtained with a minimum of readings.

The results presented below are based on the reactions of larvae stored in a saturated atmosphere for periods of four or more hours. Larvae of any instar moistened in this way aggregated in the driest zones (maximum rates of evaporation) of any groups tested within an instar. The aggregations at these maximum rates, discussed further in a following paper on responses to evaporation, proved to be very stable within an instar, and hence provided a useful standard for comparative purposes.

The distribution of larvae of any one instar could be plotted against either temperature or evaporation to obtain a satisfactory frequency distribution. Nevertheless, if all the distributions obtained from groups of one instar subjected to several gradients of evaporation in one gradient of temperature were plotted against temperature and compared, each distribution showed a distinctly different "preferred" zone of temperature. On the other hand, when these same distributions were plotted against rate of evaporation, it was seen that the larvae of the instar aggregated in one zone of evaporation, wherever this zone was placed in a wide range of temperature. This held over the range of temperature investigated, to the extent that 10° C., the lowest temperature available, did not limit the evaporation responses, while, for most instars, a temperature of about 36° C. (cf. Wellington (16)) imposed a barrier beyond which most larvae would not follow the rate of evaporation. The above statements are borne out by consideration of data for the second and sixth instars, which represent the two extremes in evaporation responses, and so give wide differences in position with respect to temperature.

If the larvae of the second instar were stored in saturated air for 4 hours or more, they exhibited a distribution with its peak at rates of evaporation of 0.13 to 0.14 cu. mm. per minute. Figure 3 shows the graphic representation of this type of distribution as obtained with four groups of larvae exposed to four different types of evaporation gradients.

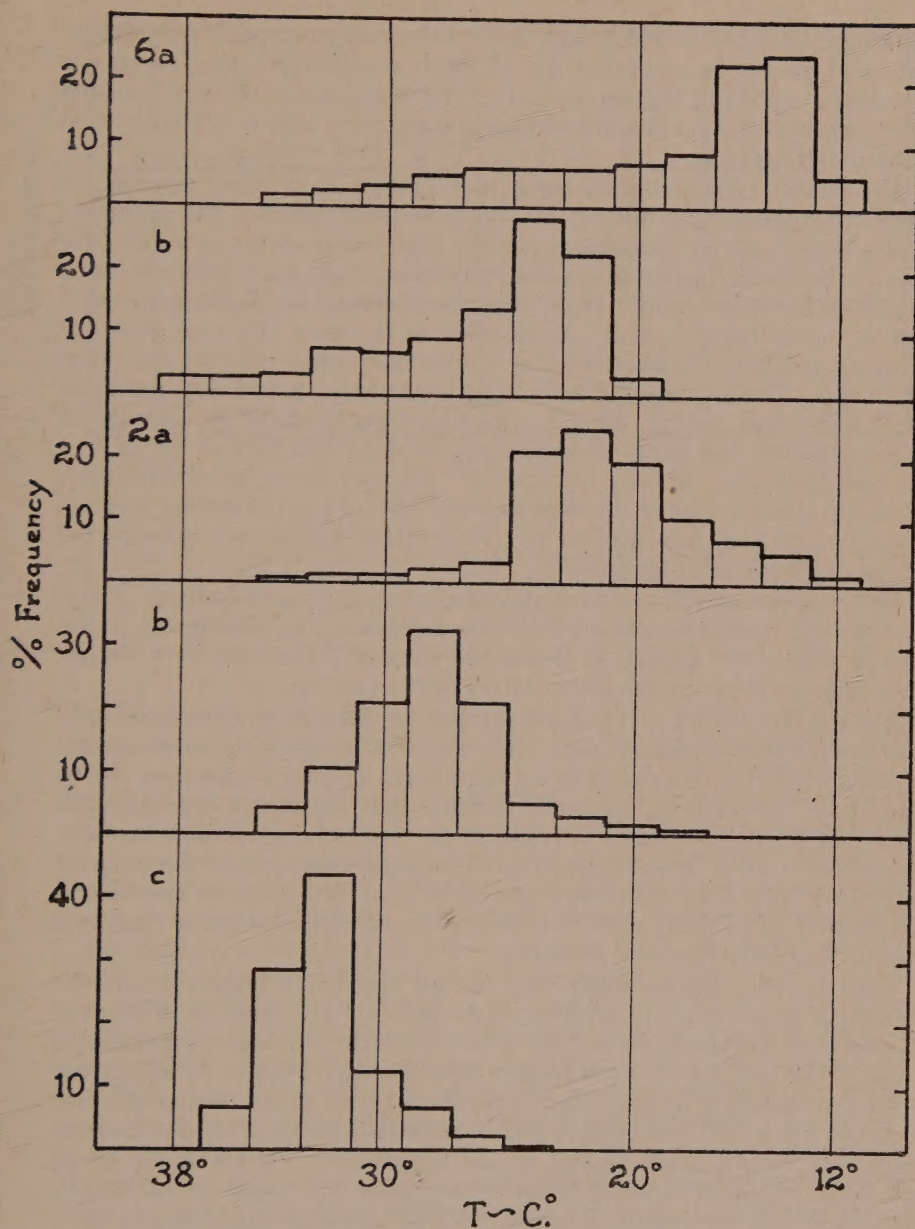


FIGURE 4. Types of distributions obtained when the numbers of larvae observed at different points in the gradients were plotted against temperature instead of evaporation: Instars 2 and 6. Instar 2: *a*: in a dry temperature gradient; *b*: in a moist temperature gradient; *c*: in a very moist temperature gradient (cf. Figure 3, *a* to *c*). Instar 6: *a*: in a moist temperature gradient; *b*: in a very moist temperature gradient (cf. Figure 5, *a* and *b*). Ordinate: percentage frequency; abscissa: temperature, Centigrade.

Each group consisted of 100 larvae, collected as they emerged from hibernacula and stored in saturated air at room temperature for four hours before being placed in the equipment. The distribution shown for each group represents the summation of four observations taken during the first hour in the apparatus.

The results obtained with these four groups show that, despite the distorted distributions produced by the different lengths of the gradients of evaporation and by the effects of very high temperature, the peak for larvae of the second instar in a moist condition is always within the same range of evaporative rates. Note that the mechanical blockage of what might be termed the "normal" distribution of larvae of this type does not necessarily result in the formation of a secondary peak right at the point of blockage. The increase in numbers may occur one or two class intervals distant from that point. This is illustrated particularly in Figures 3a and 3c. It does not seem necessary to consider this fact to be of any special significance. A large number of different types of observations have shown that the larvae, when confronted by any type of barrier, characteristically ebb and flow against it. Thus, their movements account for the type of distribution obtained.

The results noted above were also plotted against temperature. Class intervals each spanned two Centigrade degrees. The distributions are shown in Figure 4. Figure 4a shows the data of Figure 3a, with similar correspondence between the other letters of the two figures.

The significant fact which emerges when the data are plotted separately is that the actual evaporation peak for the type of larva concerned remained constant, while the temperature aggregations, although appearing to be temperature "preferences," actually indicate that the larvae aggregated in a particular temperature zone in response to a rate of evaporation, not to a temperature. Thus, it seems permissible to state that, within the range of temperature available, and below about 36° C., the larvae of the second stage showed no preference for a narrow zone of temperature as long as a gradient of evaporation was present.

This statement also holds true for all the other instars when the appropriate temperature is substituted for 36° C. This may be illustrated with reference to Figures 5a and 5b, which show the maximum evaporation rate in which groups of sixth instars aggregated. Figure 5a shows the distribution obtained by summing four observations taken during the first hour on larvae which had been stored in saturated air for 12 hours at room temperature before being placed in the apparatus. Figure 4, 6a shows these data replotted against temperature, with the peak at 14°-16° C. Figure 5b shows the results obtained with the *same* group of larvae after they were fed and stored in saturated air for 12 days at about 4° C. The gradient was very moist, yet the evaporative rate at which the peak occurred was still 0.04 cu. mm. per minute, as in Figure 5a. On the other hand, when these data were replotted against temperature (Figures 4, 6b) the peak obtained lay at 24° C., as opposed to 14°-16° C. in the previous case. These data, incidentally, indicate the relative unimportance of acclimation to temperature as opposed to the importance of evaporation in producing stable aggregations, thus stressing the fact that a temperature "preference" is more apparent than real.

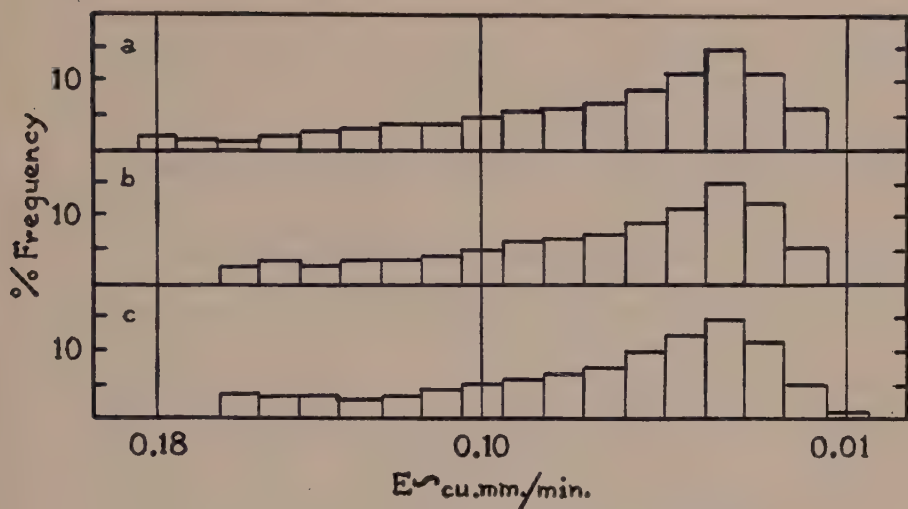


FIGURE 5. Aggregations in response to evaporation exhibited by two groups of initially moist sixth stage larvae: *a*: in a moist temperature gradient; *b*: the same group, in a very moist temperature gradient after 12 days' storage in saturated air at 4° C.; *c*: a group in an evaporative gradient held at a constant temperature of 20.6° C. Ordinate: percentage frequency; abscissa: rate of evaporation, cu. mm. per min.

The foregoing results show that, as long as a gradient of evaporation existed, it was impossible to obtain any data on the occurrence of zones of preferred temperature, in the accepted sense, for the larvae were always responding to the rate of evaporation and not to temperature at any level below about 36° C. In order to disclose any further response to temperature, it would be necessary to eliminate the evaporative gradient. This could be done only if the air in the temperature gradient were completely dry or completely saturated. Absolute dryness, if attained, would accomplish nothing, since the rate of evaporation from the larvae themselves would still increase with increasing temperature. On the other hand, it was observed that most larvae of all instars were turned back by completely saturated conditions in a gradient, and it was also found that uniformly saturated conditions at a constant temperature immobilized the insects rapidly. Nevertheless, a smaller gradient of the "bar" type was constructed using one radius of the brass disc as the floor. The sides were made by piling 2.5 × 40 cm. strips of blotter up to a height of 9 mm. The two sides were placed 5 cm. apart on the floor, and the ends were closed off with piles of blotters of similar height. A strip of plate glass served as the roof. The blotters were soaked with water and the gradient of evaporation within the enclosure was measured. This construction gave an evaporative gradient running from saturation to 0.03 cu. mm. per minute over the temperature range 10°–40° C. When the insects were placed in it, they gave the expected reactions, which were to turn back from both the saturated air at one end and the high temperature at the other, so that no additional information on temperature aggregations could be obtained. Consequently, the search for the somewhat tenuous temperature "preference" was abandoned.

Although the larvae exhibited no tendencies to aggregate in any patterns which could be construed as indicating preferred temperature ranges, they did possess a well-defined temperature sense. This was exhibited in response to radiant heat of temperatures above 36° to 38° C. by instars 2 to 6, or temperatures above about 28° C. by instar 1 (16). No extensive search was made to locate the temperature receptors, but tests with warm needles held 3 mm. from the body of a larva at different points showed that there was a definite gradient of sensitivity which increased cephalad. The head and the first four body segments were most sensitive to the radiating source. The last body segment was so insensitive that the needle had to be touched against it before a reaction was produced. This reaction was not necessarily the result of stimulation by temperature.

DISCUSSION

It has been shown that the larvae cannot be considered to exhibit a temperature preference in the accepted sense of the phrase. Since they are indifferent to temperature, below the upper limit which varies with the instar, it does not seem proper to consider the wide range below this limit as being a "preferred" zone. On the other hand, it has been shown that aggregations in response to evaporation were well-marked and consistent.

Work with aquatic organisms and with terrestrial animals living in a semi-fluid medium (Thomsen and Thomsen (12)) has demonstrated that temperature aggregations do exist. On the other hand, workers with terrestrial poikilotherms have not always considered the possible effects of atmospheric moisture on the animals observed. With few exceptions, those who have considered the role of atmospheric moisture in the complex governing the actions of animals have been content to speak of it in terms of relative humidity or saturation deficit.

If the effects of moisture are neglected altogether, difficulties may arise when attempts are made to interpret the actions of animals in gradients. For instance, Deal (3) measured the relative humidities within his apparatus, but neglected the effects of moisture in one experiment, where he reported that a braconid species moved to lower and lower temperatures in a gradient over a period of days, while a control group at room temperature died within the same time. He was unable to explain either the behaviour of the parasites in the gradient or their greater longevity. In view of the results of the present investigation, which showed that budworm larvae also moved lower in the gradients over a period of time, it would seem that the simplest explanation of the actions of the parasites is that they kept moving to lower rates of evaporation as they became desiccated. With a fixed amount of moisture in the apparatus, the lower rates of evaporation happened to be at lower temperatures. Deal also asked the question, "Why do certain insects, after being previously kept at a high temperature, have a lower preferred temperature than when previously kept at room temperature for the same length of time?" Unless the moisture conditions of storage are at saturation, the facts once more point to "desiccation" as the answer.

As noted in the introduction, rate of evaporation has been used throughout this work to demonstrate that there was a relationship between the actions of the insects and the moisture, rather than the temperature,

when these variables were presented in the form of gradients. To demonstrate any such relationship in combined gradients of two or more variables, it is necessary to show that the peak of each of a group of distributions always falls within a definite class interval of the variable related to the behaviour observed. Saturation deficiency and relative humidity are expressions of the amount of moisture in the air, but they do not take into account other factors which may affect the rate at which water vapour escapes from an evaporative surface. Thus, rate of evaporation, affected by other factors, is not directly proportional to either of the psychrometric expressions over a range of temperature. Therefore, if these expressions had been used here instead of evaporation, it would not have been possible to demonstrate that the insects, when in one particular condition, always aggregated within a definite zone of evaporation, as measured by the instrument used, over a range of temperature. This is illustrated by Table 1, which shows the positions of the peaks of the distributions of second and sixth stage larvae previously illustrated in Figures 3, 4 and 5 expressed in terms of rates of evaporation, relative humidity, saturation deficiency and temperature. These relationships were worked out from data obtained when the evaporimeter was calibrated for correlation of rates of evaporation with variable temperature and relative humidity, using solutions of sulphuric acid (cf. Wilson (17) and Buxton (2)) in a Dewar flask.

TABLE 1.—COMPARISON OF THE CONSTANCY OF THE POSITIONS OF PEAKS OF FREQUENCY DISTRIBUTIONS OF SECOND AND SIXTH STAGE LARVAE IN GRADIENTS OF MOISTURE AND TEMPERATURE, WHEN THESE DISTRIBUTIONS ARE PLOTTED AGAINST RATE OF EVAPORATION, PER CENT RELATIVE HUMIDITY, SATURATION DEFICIENCY AND TEMPERATURE

Instar	Gradient type	Evaporation, cu. mm./min.	Per cent R.H.	Saturation deficiency,* mm. Hg.	Tempera- ture, ° C.
II	Constant room T.	0.14-0.13	42.5-45.5	10.463- 9.918	20.6
	Dry T.	0.14-0.13	44.5-47.0	11.004-10.509	22
	Moist T.	0.14-0.13	50.0-52.5	14.175-13.466	28
	Very moist T.	0.14-0.13	53.0-55.5	16.762-15.870	32
VI	Moist T.	0.04	72.4-73.5	3.309- 3.613	14-16
	Constant room T.	0.04	75.0	4.549	20.6
	Very moist T.	0.04	76.0	5.371	24

* e_m obtained for saturation deficiency calculations from: Handbook of chemistry and physics, pp. 1345-1346. 22nd ed. Cleveland. 1937.

Table 1 shows that, for each instar, only the rate of evaporation gave constant values for the positions of the peaks. It is especially important to note that in the constant temperature evaporation gradient, where every part of the available area was at 20.6° C., among the three methods of expressing moisture relations, only the rate of evaporation agrees with the positions of peaks obtained later in a gradient of temperature. Thus, while there is an objection that rates of evaporation measured with different

instruments are functions of the instruments and not the animals, nevertheless, the rates measured with the instrument used provide a closer expression of the factors influencing the behaviour of the insect than do the ordinary psychrometric expressions.

It follows from the above that gradients of evaporation still exist when attempts are made to keep either a uniform relative humidity or saturation deficiency over a range of temperature, or at the points in a gradient where peaks are observed. The chief danger which arises from the neglect of this point is that, if animals move to higher temperatures in moist air than in dry air, or when they themselves are moist, one is apt to conclude that there actually is a temperature preference, which is higher at such times. As shown in Figures 3 and 4, the animals actually may be following one particular range of evaporation which has shifted to a different temperature because the moisture content of the air in the apparatus has been changed. Some indication of this type of behaviour may be seen in the description by Gunn and Cosway (5) of the actions of cockroaches which were not indifferent to moisture.

It is rather pointless to speculate too much about the relative importance of the temperature preference of terrestrial poikilotherms when these speculations are based on observations on one species. Some animals have a temperature response unaffected by evaporation, and others, such as the spruce budworm, have an evaporation preference little affected by temperature. Nevertheless, if one searches the literature while thinking in terms of evaporation rather than temperature, no very close attention is required to collect examples which strongly suggest that re-investigation of several species would considerably reduce the number of terrestrial poikilotherms now grouped in the "temperature preference" classification. There is little point in citing all such papers, but examples may be found among the lengthy lists of references collected by Uvarov (15), Fraenkel and Gunn (4) and Deal (3).

In this paper, the spruce budworm has been used as an experimental animal to demonstrate an academic point. In subsequent papers in the series, further attention is given to similar points, but also, some attention is given to items which are of particular significance to the species itself.

SUMMARY

1. There was an upper limit of temperature beyond which larvae of the spruce budworm did not travel when allowed to move freely in combined gradients of temperature and rate of evaporation. The limit for the first instar was about 28°C ., while that for instars 2 and 3 was about 36°C ., and those for instars 4, 5 and 6 ranged from 37° to 38°C .

2. Below the upper limit of temperature, larvae of any instar were indifferent to temperature presented in gradient form down to and including 10°C ., the lowest temperature available.

3. Below the upper limit of temperature, the larvae responded to the rate of evaporation, as measured by a micro-evaporimeter, and it was demonstrated that aggregations occurred within specific ranges of evaporation, regardless of where these ranges might be placed within the temperature range. Thus, the larvae of the spruce budworm cannot be considered to exhibit any temperature preference, in the accepted sense.

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THE EFFECTS OF TEMPERATURE AND MOISTURE UPON THE BEHAVIOUR OF THE SPRUCE BUDWORM, *CHORISTONEURA FUMIFERANA* CLEMENS (LEPIDOPTERA: TORTRICIDAE)

II. THE RESPONSES OF LARVAE TO GRADIENTS OF EVAPORATION¹

W. G. WELLINGTON

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INTRODUCTION

This paper is the second of a series dealing with the behaviour and activity of the spruce budworm, *Choristoneura fumiferana* (Clemens), in response to variable temperatures and rates of evaporation. In the first paper (4), it was demonstrated that larvae in apparatus containing gradients of temperature and atmospheric moisture aggregated in zones in response to evaporation rather than to temperature. The present paper describes the results of further investigations of the effects of evaporation upon aggregations of larvae observed in the laboratory.

EXPERIMENTAL MATERIAL AND APPARATUS

The majority of the experiments described in the following pages were performed during the same season as those listed in the first paper. Consequently, the insects used were obtained from the same general stock. Apparatus previously described (4) was employed during the investigations. This equipment consisted of the evaporimeter, employed to determine rates of evaporation in cu. mm. per minute, and the two types of apparatus for producing gradients of atmospheric moisture and of temperature: a linear, "bar" apparatus used to produce a graded scale of rates of evaporation at a constant temperature, and a disc-like apparatus, heated at the centre and cooled at the periphery, in which combined gradients of temperature and moisture could be established.

METHODS

As in the previous series of experiments, groups of 100 larvae were placed in the apparatus, and their positions were noted at the end of each 15-minute interval during any one hour. Positions once more were recorded first in terms of distance from a fixed point, rather than in terms of units of temperature or evaporation. Specific treatments of particular groups of larvae are described in the section outlining the observed results.

In addition to observations on groups of larvae, records were made of the behaviour of individual larvae of each of the six instars. Larvae were placed in the apparatus one at a time, and tracings were made of the paths individuals followed over fixed periods of time. An individual was kept under continuous observation during its first hour in the apparatus. Thereafter, if further information on its later behaviour was required, it was left to its own devices for one or more hours, after which another hour-long observation was made. During some observations, time marks were made on the tracings at intervals of one minute, so that the length of time spent in movement through a particular zone could be determined.

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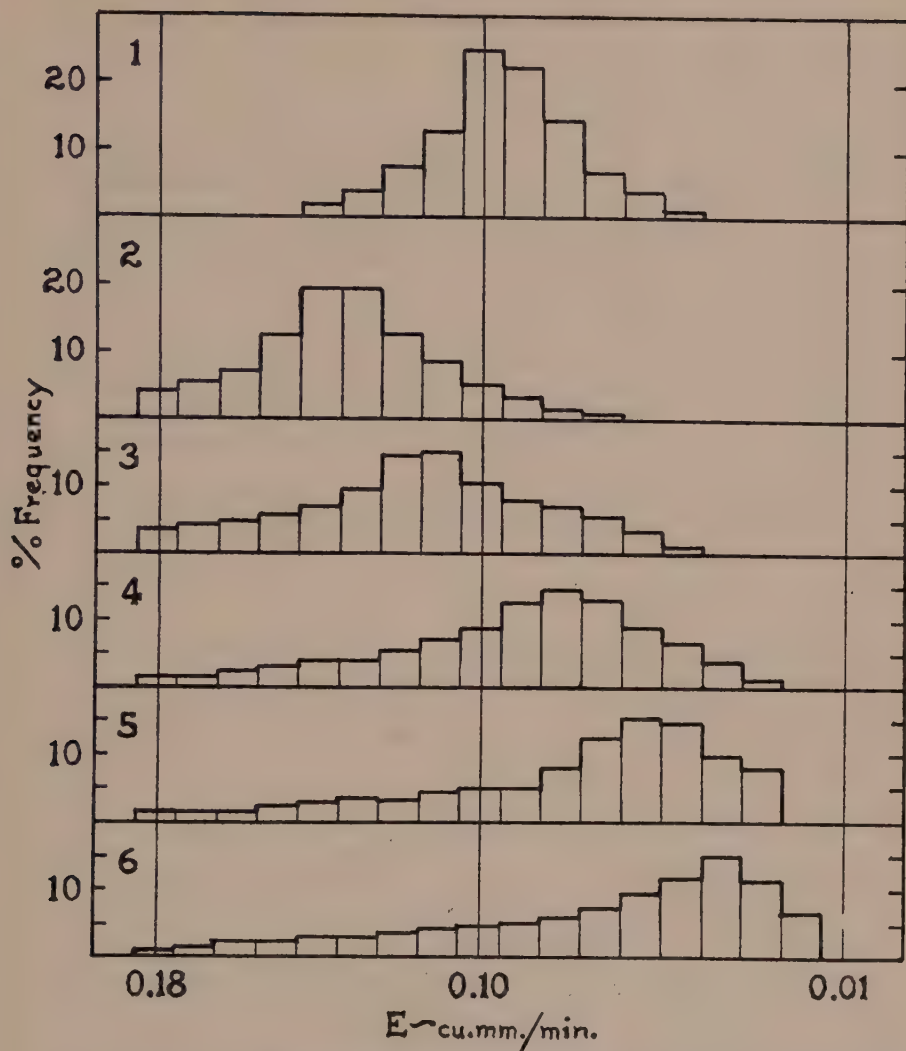


FIGURE 1. Inter-instar differences in the maximum rates of evaporation at which initially moist larvae aggregated: instars 1 to 6. Ordinate: percentage frequency; abscissa: rate of evaporation, cu. mm. per minute.

RESULTS

In the preceding paper, it was indicated that it was found possible to regulate to some extent the positions of larvae in a gradient by regulating the degree of saturation of the air to which they were previously exposed. Moreover, it was noted that, after about four hours in a saturated atmosphere, longer exposures at saturation did not result in any further movement of the peak of a frequency distribution (frequency of occurrence of individuals at definite rates of evaporation) to any higher rates of evaporation, when the larvae were finally placed in a gradient. The aggregations of larvae at these maximum rates were stable within an instar under the proper conditions, and hence the maximum rates provided useful standards for inter-instar comparisons.

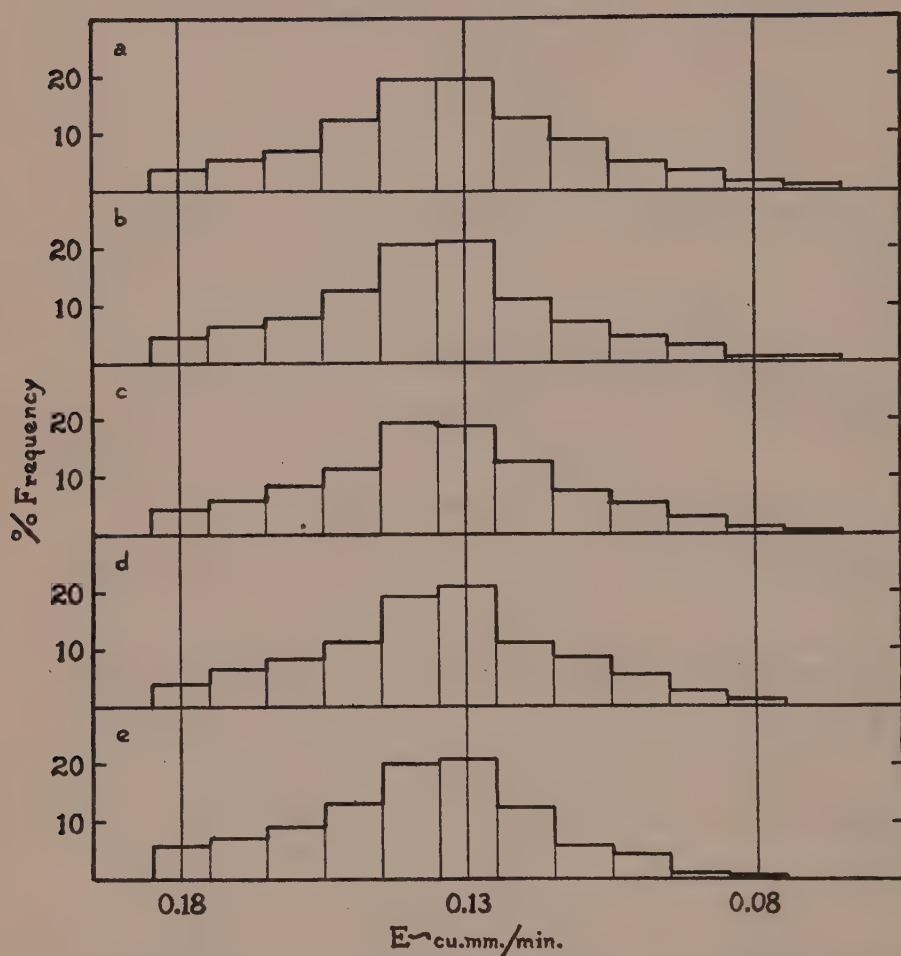


FIGURE 2. Intra-instar similarities in the maximum rates of evaporation at which initially moist larvae aggregated: instar 2. *a*: after 4 hours in saturated air; *b*: after 24 hours in saturated air; *c*: after 48 hours; *d*: after 7 days; *e*: after 14 days. Groups *a*, *b* and *c* stored at 20-21° C.; *d* and *e* at 4° C. Ordinate: percentage frequency; abscissa: rate of evaporation, cu. mm. per minute.

The differences observed among the six instars are illustrated in Figure 1. The numbers at the corners of the diagrams refer to the instar numbers. Each instar is represented by 100 larvae. Larvae of instars 2 to 6 were stored for four hours in saturated air at 20° to 21° C. before being placed in the apparatus. Larvae of the first instar were so delicate that too many were injured in attempts to remove them from a wet surface, and, since they spun webs from which it was difficult to remove them without injury when they were stored under drier conditions, they had to be tested immediately after emergence from the egg masses, without further treatment. All groups were confined in the disc-like apparatus in moderately moist air (4). The results shown for each group are the summations of four observations taken during the first hour in the gradient. The differences among instars are shown clearly in Figure 1. These differences were verified by observations on additional groups noted below.

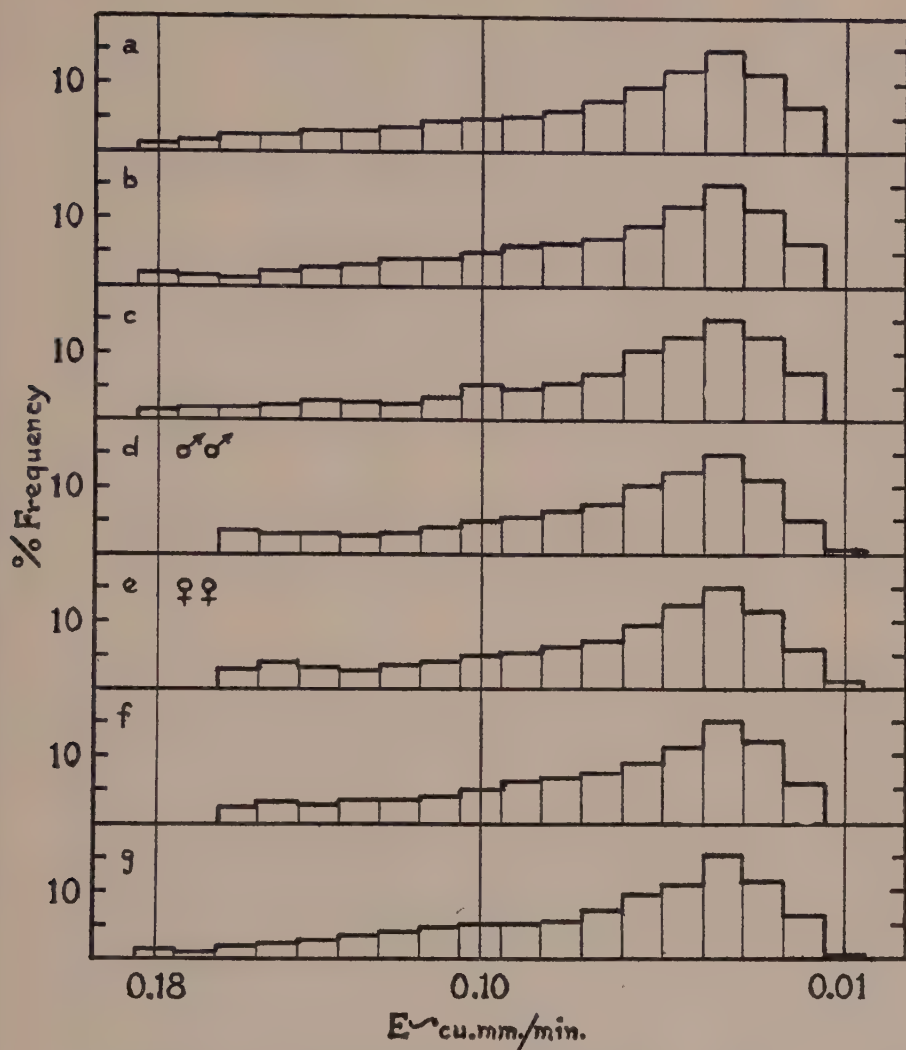


FIGURE 3. Intra-instar similarities in the maximum rates of evaporation at which initially moist larvae aggregated: instar 6. *a*: 22 days old; *b*: 32 days; *c*: 40 days; *d*: males, 32 days; *e*: females, 32 days; *f*: repeat of *b* after 12 days' storage at 4° C.; *g*: field-collected larvae, age unknown. Groups *a*, *c*, *d*, *e* and *g* previously stored for 4 hours in saturated air at 20-21° C. Group *b* stored for 12 hours. Groups *a*, *b*, *c*, and *g* observed in a moist temperature gradient; *d* and *e* in an evaporative gradient held at a constant temperature of 20° C.; *f* in a very moist temperature gradient. Ordinate: percentage frequency; abscissa: rate of evaporation, cu. mm. per minute.

Groups of larvae of each instar were observed in order to determine whether: (a) the slope or the length of the evaporative gradient; (b) the presence or absence of a temperature gradient; (c) previous treatment with saturated air for periods longer than four hours; (d) sex, or (e) age differences produced any intra-instar differences in the stable, first-hour aggregations observed for each instar.

The term "age" as used in work on instars 2 to 6, refers to the age in days calculated from the time of emergence of the second stage larvae

from hibernacula. Thus, it refers to the period of seasonal activity only, and does not include the period spanned by the preceding winter months. The ages of first stage larvae were reckoned from the time of eclosion from the eggs.

A total of 32 additional groups were observed, confirming the inter-instar differences shown in Figure 1. Sample observations from the additional series are illustrated in Figures 2 and 3. Various differences of age, sex or treatment are listed in the legends of the figures. The marked intra-instar similarities shown among the groups of the second and sixth instar larvae illustrated also were exhibited by each of the remaining instars. Thus, for larvae stored in saturated air before being placed in the apparatus, and observed during the first hour in the gradients, the results may be summarized in the following way.

Inter-instar Differences

(a) Chi-square comparisons of distributions of any two instars gave $P < 0.01$, regardless of the type of gradient employed or of similarities in the ages of the larvae. For instance, second and third stage larvae, all seven days old, still preserved the differences in response to evaporation illustrated in Figure 1.

Intra-instar Similarities

(a) Within any instar, chi-square tests of groups observed in the same type of gradient gave $P > 0.90$, regardless of differences in the ages or sex of the larvae, or in periods of previous treatment longer than four hours (see Figure 3).

(b) Within any instar, chi-square tests of distributions of groups tested in different types of gradients gave $P > 0.10$ or $P < 0.05$, depending on the types of gradients compared. The slopes of the gradients did not affect the distributions significantly. If the lengths of the gradients differed so much that $P < 0.05$ for two groups, then the region of the mode of each distribution (the instar rate) was always at the same rate of evaporation. Thus, while the lengths of the gradients affected the ends of the distributions in some instars, they did not affect the positions of the peaks of the distributions.

(c) The presence or absence of a temperature gradient affected the distributions only by affecting the lengths of the evaporative gradients used with variable temperature (see also the discussion of the upper limit of temperature in the previous paper of this series).

It has been pointed out (4) that it was only after the provision of conditions which would give constancy of the maximum rate at which larvae of an instar aggregated that any consistent differences between instars could be established. Before the correct method of previous treatment was found, several thousand records were taken and had to be discarded because they were based on populations mixed with respect to previous exposure to moisture. A specimen from these early data is included to illustrate the type of distribution obtained. This figure also is useful for interpreting what occurred in any group in later hours in the gradients. The second instar is used as an illustration.

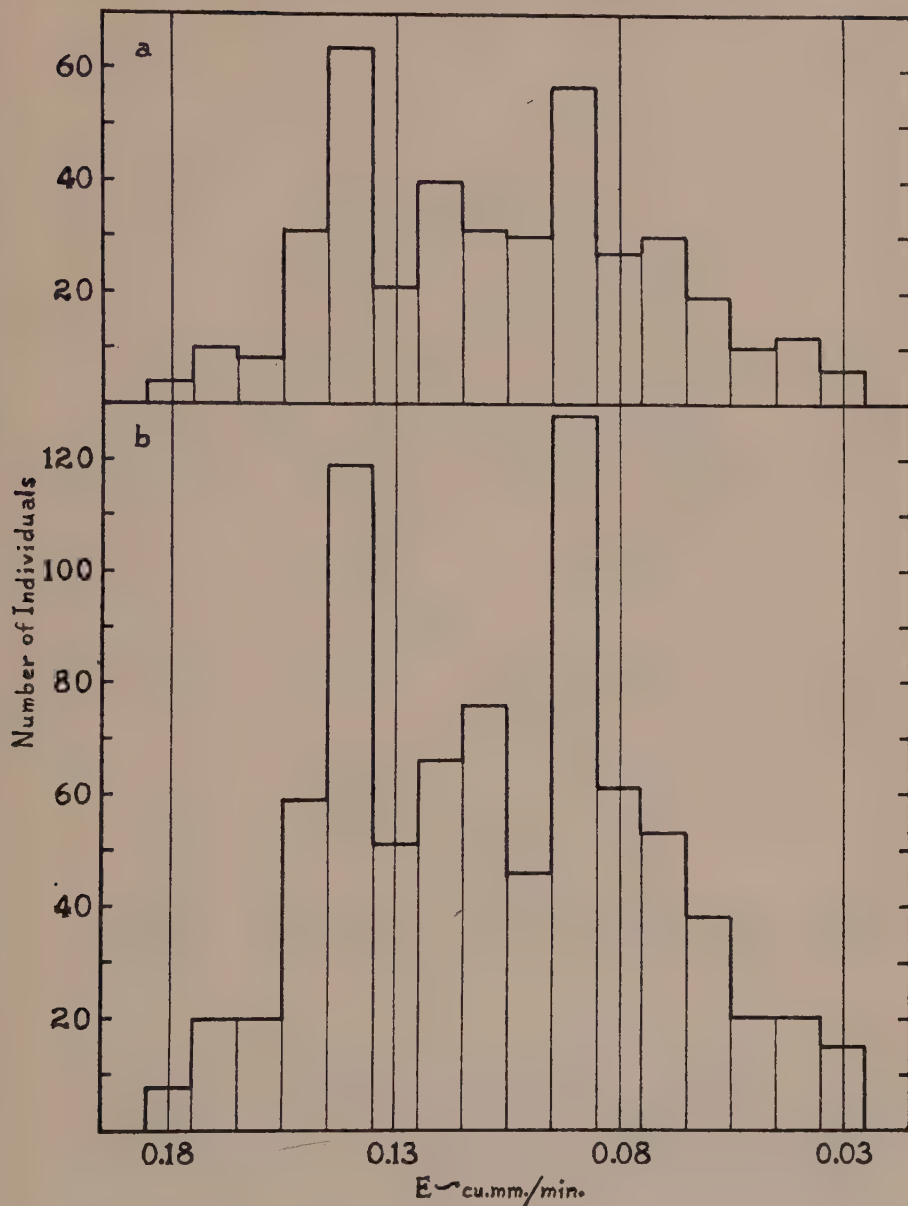


FIGURE 4. Distributions obtained when larvae were desiccated to varying degrees: instar 2. *a*: The group shown in Figure 2*a* after 3 hours in the moist temperature gradient; *b*: a group of larvae not treated with saturated air before being placed in the gradient; observation taken during the first $1\frac{1}{2}$ hours. Ordinate: number of individuals; abscissa: rate of evaporation, cu. mm. per minute.

Figure 4*b* shows a distribution exhibited by 100 freshly collected, but untreated larvae of the second stage during the first $1\frac{1}{2}$ hours of their confinement in the apparatus. This distribution was obtained by summing the results of eight observations during the period. There are three peaks, only one of which agrees with the maximum rate previously shown for the

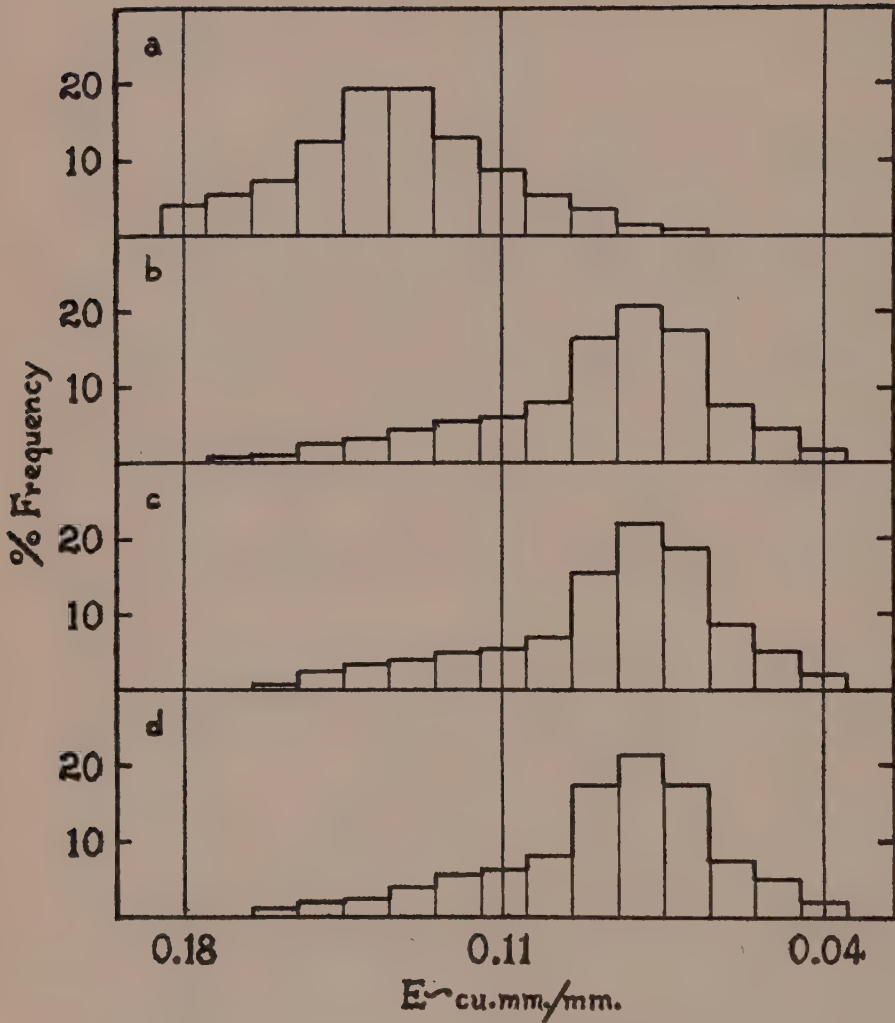


FIGURE 5. The effects of prolonged or severe desiccation upon the distribution of larvae in an evaporative gradient: instar 2. *a*: Distribution obtained in the first hour (Figure 2*a* replotted for comparison); *b*: the same group after 24 hours in the moist temperature gradient; *c*: a group previously dried for one hour in calcium chloride, observed for one hour in a moist temperature gradient; *d*: a group which had an original distribution similar to that of group *a*, but which was held for 24 hours in an evaporative gradient kept at a constant temperature of 20° C. Ordinate: percentage frequency; abscissa: rate of evaporation, cu. mm. per minute.

second instar (Figures 1 and 2). This type of distribution occurred frequently when untreated populations were used, but sometimes one or two of the three peaks were missing, or the two lower peaks were at different positions in the gradients. Such multi-modal distributions often occurred after a group had been in a gradient for a time, even if the original distribution observed had been normal for the instar. This may be illustrated further.



FIGURE 6. Tracks of larvae in a gradient of evaporation: instars 2 and 6. One-hour tracks of untreated larvae. Disc figures: rate of evaporation, cu. mm. per minute.

If second stage larvae were correctly exposed to saturated air for at least four hours, the typical distribution, such as any one of those illustrated in Figure 2, was obtained. Such distributions were stable for at least one hour and could persist for $2\frac{1}{2}$ to 3 hours, depending on the care with which the larvae were treated during previous storage (cf. 4). At the end of $2\frac{1}{2}$ or 3 hours, however, the typical distribution began to break down. Figure 4a shows a distribution obtained by summing four observations taken between hours 3 and 4 on the 100 larvae which gave the original distribution of Figure 2a. It will be seen that Figure 4a is very similar to Figure 4b, which was obtained from untreated larvae during their first hour in the apparatus.

During the next 12 hours larvae were in the apparatus, almost any type of distribution could be obtained. The only thing common to the many types was that the maximum evaporation peak observed was never

higher than the original maximum noted for the instar. During the last 6 hours of a 24-hour period, a new, stable distribution occurred at a considerably lower level in the gradient. The change which occurred over the 24 hours may be seen in Figure 5.

Figure 5a shows the same group illustrated in Figure 2a, re-drawn here for comparison. Figure 5b shows the distribution exhibited by this group after 24 hours in the apparatus. Both of these may be compared to the 3 to 4 hour distribution, an intermediate type, shown in Figure 4a. Figure 5c shows the distribution obtained during the first hour from four observations on a group of 100 larvae which had been dried in calcium chloride for one hour before being placed in the apparatus.

Some further information on the behaviour of larvae in gradients was obtained by observing individual larvae for hour-long periods. Figure 6 shows examples of two types of one-hour tracks which individual larvae followed in a concentric evaporation gradient. Each track begins with a dot and the direction of the arrow at the end of the track shows the direction in which the larva was moving at the end of the observational period. Numbers at the dots refer to the instar numbers. It must be emphasized that the types of paths shown for each instar were not peculiar to the instar, but might be exhibited by individuals of any instar. Thus, a sixth stage larva might, on occasion, follow a convoluted path much like that of the second stage shown in the figure. Moreover, since these were individual larvae, the "preferences" indicated did not necessarily agree with the group "preferences" obtained for the instars concerned.

Figure 7 shows two tracks obtained from one individual of the third instar, while it was in two different states. Track (a) shows the path of the larva during its first hour in the gradient. The larva was removed from the foliage on which it was feeding and placed directly in the gradient without any special treatment. Track (b) shows the path taken by the larva after it had been picked up while moving to a low rate of evaporation at the end of track (a) and returned to the original starting point. Figure 8 presents the information of Figure 7 in a different manner. The histograms indicate the time, in minutes, spent within the different zones of evaporation during the course of each of the consecutive, hour-long periods.

It was found that light, food and foreign matter could be used to draw larvae of different instars from their particular zones of aggregation. Under certain temperature conditions (3), all stages reacted positively to diffuse light, and could be drawn into a zone of even completely saturated air by darkening the rest of the gradient. Within this zone, they soon became immobilized and so were trapped in it. They also could be drawn into areas where rates of evaporation were excessively high by the same method. In the room temperature evaporation gradient, larvae of all instars were held by the light in a high rate of evaporation until they became moribund from desiccation. In the combined temperature and evaporation gradients, larvae could not be drawn much above the 36° isotherm by light (3) or any other factor. It should be emphasized that consistent responses could be obtained only when diffuse light was employed to draw larvae along or around apparatus (cf. 3).

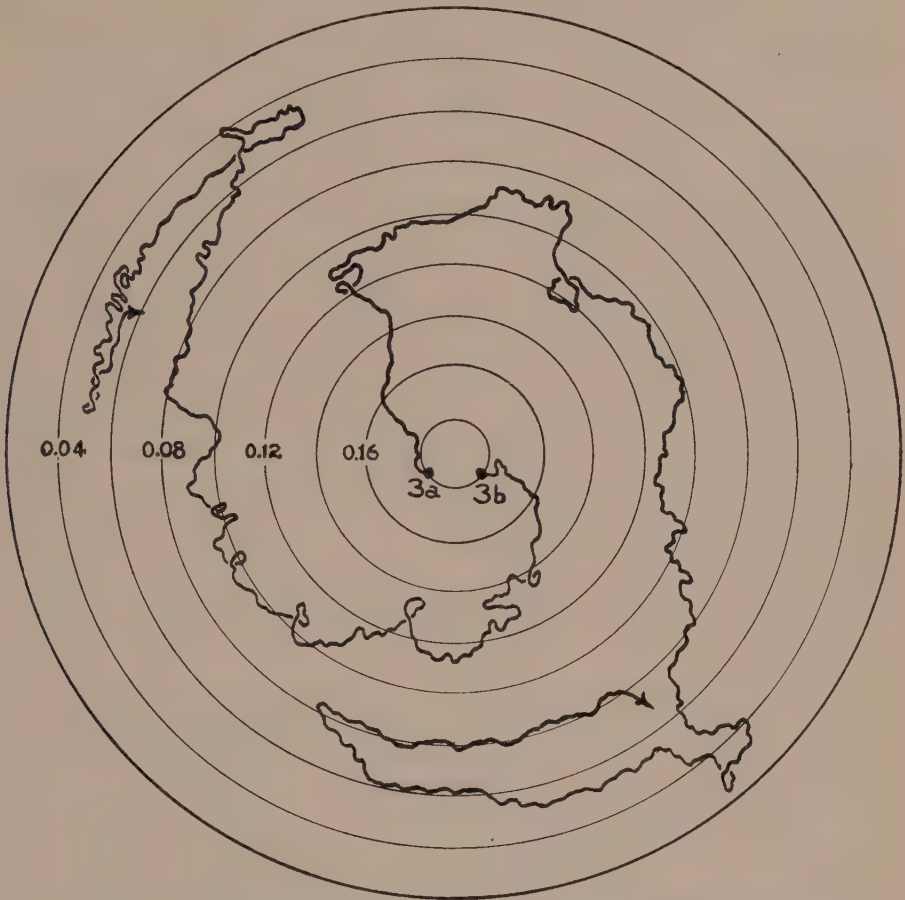


FIGURE 7. Tracks of larvae in a gradient of evaporation: instar 3. *a*: one-hour track of freshly-collected, untreated larvae; *b*: the same larva, collected while moving at a low rate of evaporation and observed for an additional hour. Disc figures: rate of evaporation, cu mm. per minute.

Instars 2 to 6 responded to the presence of food in the apparatus when they became sufficiently hungry. Individual needles were used so that the larvae could not climb off the floor of the disc as they could have done if twigs had been used. The larvae did not move directly to the needles, but, if they encountered them, they remained by them until the needles began to dry.

DISCUSSION

It has been shown that suitable previous treatment revealed differences in the rates of evaporation at which larvae of the six instars aggregated. The groups in these zones consisted of insects conditioned by saturated air to a point at which their retained water apparently was at its possible maximum. In this condition, they reacted to dry air (cf. Bentley, 1), but exhibited instar differences in the maximum rates of evaporation to which they would move.

Despite the fact that these differences were well-marked, as illustrated in Figure 1, and consistent, as noted in the preceding section, it also has been shown that they were short-lived, existing for a maximum period of three hours. Within this interval, intra-instar similarities could be demonstrated. After this interval, multimodality, as illustrated in Figure 4, appeared, and intra-instar comparisons became impossible.

The frequent occurrence of the type of distribution shown in Figure 4b when untreated populations were used might have been interpreted as indicating the presence of two or more physiological races differing in their responses to evaporation (cf. Wilkes, 5). The facts that only one of the peaks ever occurred in the same place and that, occasionally, regular distributions were obtained did not lend too much weight to this hypothesis.

The observations illustrated in Figures 4 and 5, when repeated for each instar, showed conclusively that the positions of peaks of distributions were affected by the degree of desiccation of the larvae. Thoroughly moist larvae aggregated at the instar rates previously illustrated. Larvae which were partially desiccated reversed their reaction to dry air and tended to aggregate at lower rates of evaporation. The drier the larvae, the lower the rates of evaporation at which they aggregated, and the sooner they collected in such zones. This last point is emphasized by Figure 5c, showing larvae which had been dried by calcium chloride before being placed in the apparatus.

Thus, the multimodality exhibited for several hours by larvae which were initially very moist or mixed in this respect (Figure 4) was brought about by larvae of varying degrees of desiccation aggregating in different zones at the same instant. Observations on individual larvae, such as those illustrated in Figures 7 and 8, showed that any one larva might recover from the effects of desiccation by moving to a zone of lower evaporation, and later return to the original zone or to an intermediate one (see also Figure 6). As the time of exposure in the gradient was prolonged, and the larvae remained unwatered and unfed, the highest rate of evaporation to which these larvae returned for any length of time became lower and lower. Thus, the larvae were gradually forced to lower and lower zones in the gradient. Eventually, this resulted in a recurrence of stable distributions which lacked additional peaks, such as that shown in Figure 5b. Since individual larvae exhibited the same sort of behaviour as that shown by a group, it was not necessary to call upon the concept of physiological races to assist in the interpretation of the movements of the groups. It was thought for a time that there was some evidence of differentiation among individual larvae, since it was noticed that some individuals observed for one hour remained in a narrow zone, while others fluctuated as described above. However, it was found that any one larva might exhibit both types of behaviour, so the problem was left at that point. If races exist, they did not indicate their presence by exhibiting different responses to evaporation in any of the experiments reported here.

It should be emphasized that the aggregations at the low rates illustrated in Figure 5 were not at the minimum rates for the instar. About all that may be said concerning the lowest rates to which larvae would move is that, if larvae were desiccated sufficiently, they all eventually approached,

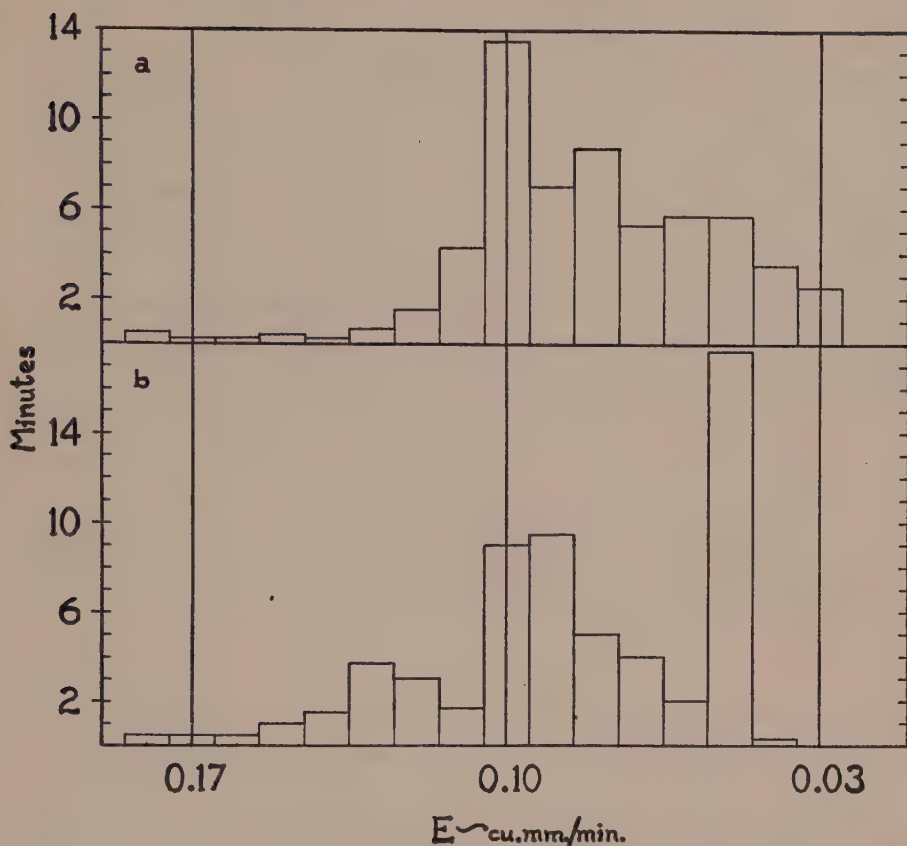


FIGURE 8. Quantitative presentation of the tracks of the third instar larva shown in Figure 7. The time, in minutes, spent at different rates of evaporation during each hour in the gradient. *a*: during the first hour; *b*: during the second hour.

but seldom entered, the zone of completely saturated air. There were no instar differences in this respect, although, as shown in the preceding illustrations, the later instars responded initially to rather low rates.

Tracks such as those illustrated in Figures 6 and 7, particularly when translated into terms of the amounts of time spent in restricted areas (Figure 8), showed some features of the mechanism of the reaction to evaporation. For instance, Figure 8 shows that an individual larva spent considerably more time at definite rates of evaporation, the levels of which varied with the condition of the larva. Spruce budworm larvae of instars 2 and 3, and larvae of the first instar lacking a suitable substrate for spinning, moved almost constantly while in the apparatus, differing in this respect from some species of insects commonly used in preferendum studies. Observations of their movements revealed a number of reasons for the excess time spent in restricted zones. The reasons are listed below.

Outside the zone, movement was rapid, and orientation was direct, with the path leading across the iso-lines of the gradient. As the zone was approached, and, often, after it had been entered, a larva appeared to move at a slower rate. Although the actual speed of movement was not decreased

and sometimes, actually increased, more time was spent in covering a given distance, because there were more frequent momentary hesitations during which tests of the surroundings were made by lateral body movements. In addition to these comparisons of the intensities of stimulation, there was a good deal of "virtual inactivity" (Gunn and Pielou, 2), during which the larva kept moving, but turned so sharply and so continuously that it covered very little territory. Akin to this type of behaviour was an increase in the actual amount of turning shown by some individuals. The net result was that the frequent hesitations, "virtual inactivity" and increased amount of turning more than counterbalanced the fairly direct movement outside the zone. Hence, more time was spent at the rate of evaporation within the zone than at any other rate. At the upper and lower limits of evaporation, beyond which a larva did not move, a sharp, "avoiding" reaction occurred.

Larvae of instars 4 to 6 frequently exhibited the same behaviour patterns as those noted above. In addition, actual cessation of locomotion sometimes occurred when the larvae reached zones in which the instar evaporation rates prevailed. Larvae, on entering these zones, might remain stationary, except for head movements, five or ten minutes at a time. This tendency to stop within the zone of the instar rate (or at lower rates, if the larvae were desiccated) was most marked among larvae of the sixth instar.

SUMMARY

1. Larvae conditioned to saturated air showed inter-instar differences in their responses to rates of evaporation during their first three hours in a gradient.

2. Different groups of similarly conditioned larvae of any one instar showed similar responses to evaporation, regardless of differences in the ages or sexes of the larvae, or of differences in the slopes of the gradients. The lengths of the gradients affected distributions of larvae only by restricting the movements of individuals on the outer fringes of a group.

3. The drier larvae became, the lower the rates of evaporation at which they aggregated. Partial, temporary recoveries from desiccation could be observed at the lower rates, but, eventually, all larvae approached, but did not enter, the zone of saturated air.

4. The mechanism of the response to evaporation contained several behaviour elements. More time was spent within a zone of evaporation than outside it because, within the zone, time spent in increased amount of turning, "virtual inactivity" and more frequent hesitations for tests of the surroundings more than counterbalanced time spent in direct movements outside the zone.

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EPIDEMIOLOGY OF RUST IN WESTERN CANADA AS INFLUENCED BY THE INTRODUCTION OF STEM-RUST RESISTANT VARIETIES¹

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INTRODUCTION

The recent introduction of rust-resistant varieties of wheat and oats in the western plains region of the United States and Canada has gone far towards eliminating one of the major hazards to cereal production in that area. It is generally recognized that since the introduction of these varieties rust losses have been drastically reduced. As might have been expected, the substitution of resistant for susceptible varieties over an area of several million acres has profoundly affected the pattern of rust development, particularly in the northern part of the western plains region. The number of stem-rust spores present in the air over Western Canada during the summer months has diminished. The spread of stem rust westward and northward into the areas of Western Canada where susceptible varieties of wheat and oats are still grown has been restricted. Significant changes have taken place in the relative prevalence of races of stem rust of oats and leaf rust of wheat. These secondary effects, resulting from the introduction of rust-resistant varieties, are considered to be of sufficient importance from both the economic and scientific viewpoints to warrant detailed examination. It is with this question that the present paper deals.

In 1937, small amounts of seed of stem-rust resistant varieties of wheat and oats were released to farmers in the area of Western Canada subject to rust attack. With the seeding of the 1939 acreage, the change-over from susceptible to resistant varieties was all but completed and, in that year, almost 80 per cent of the wheat acreage and much of the oat acreage in this area was sown to stem-rust resistant varieties. In this study, therefore, the year 1938 and previous years are placed in the period in which susceptible varieties were generally grown, and the year 1939 and subsequent years are placed in the period in which stem-rust resistant varieties held the predominant position.

EXPERIMENTAL METHODS

The effect of the substitution of stem-rust resistant varieties for susceptible ones on the epidemiology of cereal rusts in Western Canada has been determined by comparing stationary spore-trap data, uniform rust-nursery data, and physiologic-race survey data, over a period of several years prior to and subsequent to the general introduction of resistant varieties in 1939; and by comparing the performance of a susceptible variety, Marquis, and a resistant variety, Regent, in uniform field tests throughout this area.

All the spore-trap data, the rust-nursery data, and the yield data were analysed statistically to determine the significance of the differences between the various sets of data compared. In all the statistical analyses carried out, analysis of variance methods were employed.

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INFLUENCE OF CHANGE FROM SUSCEPTIBLE TO RESISTANT VARIETIES ON THE PREVALENCE OF AIR-BORNE INOCULUM OVER MANITOBA

Stationary spore-trap slides were exposed each summer from 1926 to 1946 at three stations in Manitoba—Morden, Brandon, and Winnipeg. The numbers of stem and leaf-rust spores caught on the slides were recorded. Table 1 gives, for the Morden and Brandon stations, the total numbers of stem-rust spores caught on a square inch of slide during each 10-day period in June and July over an 8-year period, 1931 to 1938, when susceptible varieties were grown, as well as over an 8-year period, 1939 to 1946, when stem-rust resistant varieties predominated. It also gives, for the Winnipeg station, similar data for the 7-year periods, 1932 to 1938 and 1940 to 1946. This table shows that at all three stations the spore counts were much lower for the period when resistant varieties predominated than for the period when susceptible varieties were grown. For each station the reductions in spore counts were statistically significant. The table further shows that the disparity between spore counts for the two periods was much greater towards the end of the season, at which time locally produced inoculum predominated in the air, than at the beginning of the season when wind-borne inoculum from more distant infection sources predominated.

TABLE 1.—THE TOTAL NUMBER OF STEM-RUST SPORES INTERCEPTED ON 1 SQ. IN. OF SLIDE AT MORDEN, BRANDON, AND WINNIPEG DURING 10-DAY INTERVALS IN JUNE AND JULY FOR PERIODS PRIOR TO AND AFTER THE GENERAL INTRODUCTION OF STEM-RUST RESISTANT VARIETIES IN 1939

Exposure interval	Morden		Brandon		Winnipeg	
	8-year period		8-year period		7-year period	
	1931-1938	1939-1946	1931-1938	1939-1946	1932-1938	1940-1946
June 1-10	16	2	35	8	19	13
June 11-20	141	19	49	23	62	18
June 21-30	233	94	98	71	844	331
July 1-10	2,086	971	392	214	607	426
July 11-20	29,107	7,057	8,015	551	6,352	2,026
July 21-30	178,471	24,072	57,288	2,814	71,480	5,158
Total spores	210,054	32,215	65,877	3,681	79,364	7,972

The total numbers of leaf-rust spores caught on each square inch of slide, recorded in 10-day exposure intervals during June and July, are given in Table 2 for the two 8-year periods under review. The data presented in this table show that with the introduction of stem-rust resistant varieties there was no reduction in air-borne leaf-rust inoculum. In fact, at the Brandon station, there was a significant increase in the numbers of leaf-rust spores caught on the slides following the introduction of stem-rust resistant varieties. At the other two stations there was no significant difference in the numbers of spores caught on the slides for the 8-year periods before and after the introduction of stem-rust resistant varieties. This result may be attributed to the fact that none of the stem-rust resistant varieties was very highly resistant to leaf rust. One of these varieties, Thatcher, which since 1939 has occupied from one-third to one-half of the

TABLE 2.—THE TOTAL NUMBER OF LEAF-RUST SPORES INTERCEPTED ON 1 SQ. IN. OF SLIDE AT MORDEN, BRANDON, AND WINNIPEG DURING 10-DAY INTERVALS IN JUNE AND JULY FOR PERIODS PRIOR TO AND AFTER THE GENERAL INTRODUCTION OF STEM-RUST RESISTANT VARIETIES IN 1939

Exposure interval	Morden		Brandon		Winnipeg	
	8-year period		8-year period		7-year period	
	1931-1938	1939-1946	1931-1938	1939-1946	1932-1938	1940-1946
June 1-10	16	127	58	126	35	30
June 11-20	889	267	137	235	578	280
June 21-30	1,610	1,241	604	1,237	1,058	2,939
July 1-10	11,854	9,271	781	2,676	1,186	2,552
July 11-20	53,160	39,738	1,315	9,615	9,342	9,645
July 21-30	92,300	46,728	11,711	22,479	29,201	14,552
Total spores	150,829	97,372	14,606	36,368	41,400	29,998

wheat acreage in Manitoba and eastern Saskatchewan, is much more susceptible to leaf rust than Marquis, and is quite as susceptible as Ceres to this rust. It was these two varieties that Thatcher largely displaced. Two other important stem-rust resistant varieties, Regent and Renown, although very much more resistant to leaf rust than Marquis and Ceres, are moderately susceptible to certain races of leaf rust and have in some years carried considerable leaf-rust infection. Moreover, with the exception of Ajax, none of the stem-rust resistant varieties of oats grown in this area possesses any resistance to crown rust. Ajax has some mature-plant resistance to certain races of crown rust. With the introduction of these varieties of wheat and oats, therefore, there was no marked change in air-borne leaf-rust inoculum.

EFFECT OF GROWING RESISTANT VARIETIES IN THE RUST AREA ON THE NORTHWARD AND WESTWARD SPREAD OF RUST

Uniform rust nurseries in which both resistant and susceptible varieties were grown have been located over a period of years at several places in Western Canada. Rust readings have been taken on these varieties every year since 1939. The longest continuous period for which rust readings are available, prior to the introduction of rust-resistant varieties, is a 5-year period from 1925 to 1929, inclusive. This period is fairly representative of the rust conditions that prevailed during the period that susceptible varieties were grown, for, according to Craigie (1), it includes one heavy rust year, 1927; one medium rust year, 1925; and three light rust years, 1926, 1928, and 1929. A comparison of rust readings on the susceptible varieties Marquis and Little Club in the uniform rust nurseries for this period with rust readings on the same varieties in rust nurseries during the 5-year period 1942 to 1946, shows that stem-rust infection on these varieties was much heavier in Western Canada during the period in which stem-rust susceptible varieties predominated (Table 3). Furthermore, the farther northward and westward the areas were located the greater was the difference between the highest rust readings for the two periods under consideration. These findings indicate that the northward and westward spread of stem rust was appreciably less during the latter period.

TABLE 3.—THE PERCENTAGES OF STEM RUST OF WHEAT FOR THE PERIOD 1925 TO 1929 AND THE PERIOD 1942 TO 1946, ON SUSCEPTIBLE VARIETIES GROWN IN UNIFORM RUST NURSERIES IN WESTERN CANADA

Area	Average infection for period (per cent)		Highest infection for period (per cent)	
	1925-1929	1942-1946	1925-1929	1942-1946
Eastern Manitoba	68	36	90	55
Mid-western Manitoba	66	25	95	60
Eastern Saskatchewan	22	17	80	60
Mid-western Saskatchewan	27	1	68	10
Western Saskatchewan	13	Trace	68	Trace
Eastern Alberta	1	Trace	Trace	Trace

EFFECT OF RESISTANT VARIETIES ON THE PREVALENCE OF DIFFERENT RACES OF RUST

Collections of stem rust of wheat (*Puccinia graminis Tritici* Erikss. & Henn.), leaf rust of wheat (*P. triticea* Erikss.), crown rust of oats (*P. coronata* Corda var. *Avenae* Erikss. & Henn.), and stem rust of oats (*P. graminis Avenae* Erikss. & Henn.) have been made and identified each year since 1925, from many separate localities in the Prairie Provinces of Western Canada.

The introduction of stem-rust resistant varieties has apparently had no effect on the wheat stem rust physiologic-race complex in Western Canada. The relative proportions of the races have not changed materially since the general advent of resistant varieties. However, a new race of stem rust, 15B, that can heavily attack the presently grown resistant varieties was found in one locality in Manitoba in 1946.

With respect to leaf rust of wheat the situation is somewhat different. The varieties Regent and Renown, during the first few years after their release, proved to be at least moderately resistant to leaf rust. But during the past several years new races of leaf rust, such as race 128, and certain biotypes of races 5 and 15, have appeared and increased. These races, reported by Johnson and Newton (2), are able to attack Regent and Renown heavily, and both varieties have carried heavy infections in many localities in Western Canada in the three years, 1944 to 1946.

A very marked change has taken place in the relative prevalence of the races of stem rust of oats since the new stem-rust resistant varieties were distributed. Before the varieties Vanguard (released in 1937) and Ajax and Exeter (released in 1943) became the predominant varieties in the rust area, the common races of oat stem rust, 1, 2, and 5, to which these varieties are all highly resistant, comprised over 90 per cent of all the races isolated from collections of stem rust of oats made in Western Canada. Races 8, 10, and 11, to which these three varieties are susceptible, were only rarely collected and in some years were not even represented among the races isolated. In 1943, a notable increase in the prevalence of races 8, 10 and 11 was apparent, and each year since this increase has continued. In 1945 and 1946, these three races comprised 33 per cent of the oat stem-rust isolates obtained from Western Canada.

No change has occurred in recent years with regard to the relative prevalence of the races of crown rust of oats present in Western Canada.

THE EFFECT OF THE GROWING OF RESISTANT VARIETIES ON THE YIELD OF SUSCEPTIBLE VARIETIES IN THE RUST AREA

Within the rust area of Western Canada (Manitoba and eastern Saskatchewan), the acreage seeded to rust-susceptible varieties has been negligible since resistant varieties became generally obtainable, and no dependable records are available concerning the yields of susceptible varieties in commercial fields. However, both resistant and susceptible varieties have been grown each year in experimental plots located in several places in each of the three Prairie Provinces of Western Canada.

During the 5-year period (1934 to 1938) just prior to the introduction of stem-rust resistant varieties the yield of Regent wheat in experimental plots at four stations in the rust area (Morden, Brandon, Portage la Prairie, and Gilbert Plains) exceeded the yield of the rust-susceptible variety, Marquis, by 12.1 bushels per acre. But for the 8-year period (1939 to 1946) after resistant varieties were introduced Regent out-yielded Marquis at these same four stations by only 7.2 bushels per acre. The yield differences between Marquis and Regent were statistically significant for both periods under review. As compared with the performance of Regent, the showing of Marquis during the latter period improved by some 4.9 bushels per acre. The improved showing of Marquis during this latter period was most probably due to the reduction of rust inoculum in the rust area brought about by the displacement of susceptible varieties by resistant ones.

That the differences in yield of Regent and Marquis in the rust area for the periods under review were due to the differential response of these varieties to stem-rust, rather than to any inherent difference in their yielding ability, was indicated by their yields in the areas of Western Canada where stem rust infection is usually light or absent. During the 8-year period 1939 to 1946, stem rust on Marquis and other susceptible varieties in these areas (western Saskatchewan and Alberta) was negligible. The average yields of Regent and Marquis for this period at four stations (Scott, Saskatchewan, and Lethbridge, Edmonton, and Lacombe, Alberta) were almost identical, namely, 36.6 bushels per acre for Regent and 36.8 bushels per acre for Marquis.

DISCUSSION

From the inferior yield performance of Marquis as compared with the yield of Regent in the rust area for the period since rust-resistant varieties became predominant, it may be concluded that the extensive acreage covered by resistant wheat varieties affords only partial protection to susceptible wheats in this area.

The principal sources of the air-borne stem-rust inoculum that is present in Western Canada during the summer months are susceptible varieties of wheat, oats, and barley, and certain wild grasses. With the introduction into the rust area of stem-rust resistant varieties of wheat, a notable reduction in the amount of air-borne inoculum took place. There still remains, however, an important reservoir for stem-rust inoculum in the extensive areas occupied by susceptible grasses, such as wild oats (*Avena fatua*), wild barley (*Hordeum jubatum*), *Agropyron* spp., and a number of other grasses which harbour stem rust, as well as susceptible varieties of

barley and certain varieties of oats that are resistant to some but not all of the races of stem rust present throughout the prairie region. Had these susceptible hosts not been so numerous and widely distributed throughout the rust area of Western Canada and the United States, a greater reduction in air-borne stem-rust inoculum than that indicated by the spore counts would probably have taken place following the introduction of resistant varieties.

The data presented in Table 1 show that in the period since the introduction of rust-resistant varieties in Western Canada there has been a considerable reduction in the numbers of stem-rust spores appearing in southern Manitoba in the early part of June. This is probably due to the fact that fewer spores are now being produced in the adjacent spring wheat area of the United States, where the crop consists mainly of resistant varieties. But in southwestern United States and northern Mexico, the area in which stem rust overwinters, susceptible varieties are still being grown. From this area stem rust spreads northward in the spring into the winter wheat belt of Kansas, where susceptible or partially susceptible varieties predominate. By late June the wave of stem-rust infection has extended farther north into the spring-wheat area of United States and Canada. It is apparent, therefore, that the amount of stem-rust inoculum appearing in southern Manitoba in the early part of June each year, as well as the physiologic races represented in it, are determined by the host plants present in the areas in which stem rust overwinters, and in which it develops as it spreads northward. As long as susceptible hosts are present in these areas it may be expected that stem-rust spores will continue to appear in the rust area of Western Canada and there infect susceptible cereals and grasses.

The reduction in prevalence of common physiologic races of rust due to the introduction of resistant varieties affords, by reduced competition, a greater opportunity for development and spread of virulent physiologic races that are already extant or that may be produced in nature by hybridization or mutation. That this occurs is indicated by: (1) the appearance and increase in prevalence of races such as race 128 of leaf rust of wheat following the introduction of Renown, Regent, and other wheats possessing resistance to many other races of leaf rust; (2) the increase in prevalence of races 8, 10, and 11 of stem rust of oats following the introduction of varieties, such as Vanguard and Ajax, which possess resistance to the common races of oat stem rust; (3) the appearance in Canada in 1946 of race 15B of wheat stem rust, a virulent race, first reported by Loegering and Stakman (3) in the United States in 1942.

The results of the present epidemiological study show that important changes are continually taking place in the physiologic race complex of at least some of the cereal rusts present in Western Canada. New races that are capable of attacking some of the new rust-resistant varieties have appeared, and virulent races that were quite scarce and unimportant in former years have increased in prevalence until they threaten to nullify much of the work of the plant breeders. These changes have necessitated modifications in the plant breeding programme to counteract the effects of the existing race complex. Since further changes in the race complex

of the cereal rusts may be expected to occur in the future, it will be necessary to continue epidemiological studies in Western Canada so that timely adjustments to meet these changes may be made in the breeding programme for rust resistance.

SUMMARY

1. Data obtained from spore-trap exposures, uniform rust nurseries, physiologic-race surveys, and yield tests have been analysed in an attempt to appraise the effect on the epidemiology of cereal rusts in Western Canada of introducing rust-resistant varieties of cereals in the rust area of the mid-western United States and Canada.

2. The substitution of stem-rust resistant varieties for susceptible ones has reduced the amount of stem-rust inoculum present in the air over Western Canada during the growing season, but has had no appreciable effect on the amount of air-borne leaf-rust inoculum.

3. Stem rust of wheat apparently does not now spread as far northward and westward in Western Canada as it did before resistant varieties were generally grown.

4. Since the introduction of resistant varieties, a definite change has taken place with respect to the relative prevalence of the various physiologic races of stem rust of oats and leaf rust of wheat present in the rust area. The races of these rusts that are more virulent towards the newly introduced cereal varieties have increased in prevalence. The introduction of resistant varieties has not appreciably affected the complex of the physiologic races of stem rust of wheat and crown rust of oats in Western Canada. However, a new race of stem rust, 15B, capable of infecting the varieties of wheat that are now being grown throughout the rust area, was found in one locality in Manitoba in 1946.

5. Susceptible varieties within the rust area, although afforded a certain amount of protection by the surrounding acreage of resistant varieties, are still subject to the rust hazard, as was demonstrated in yield tests.

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THE EFFECT OF FLOODING ON EMERGENCE OF FORAGE CROP SEEDS¹

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Time of seeding is one of the major problems associated with the successful establishment of forage crops on low lying spring flooded lands in Western Canada. Depending on the length of the flooding period, it may not be possible to seed such areas until June or even early July. Seeding at this time is extremely hazardous as the following period is frequently hot and dry and, consequently, seedlings may not survive. Other seeding dates are early fall or late fall just prior to freeze-up. The latter date is most successful on areas not subjected to early spring flooding. This time of seeding would be preferable on spring flooded land providing the forage crops used were able to emerge sufficiently to produce a satisfactory stand following the flooding period.

In a previous paper³ results were reported on the emergence of Reed canary grass seed following periods of flooding from 7 to 63 days. The data from this greenhouse experiment showed a slight decline in emergence as the length of the flooding period increased. The number of seeds that emerged following the longest flooding period was still considerable, and would be enough to result in a satisfactory stand under field conditions. The ability of Reed canary grass seed to remain viable under water for relatively long periods and emerge following drainage, prompted the authors to investigate the effect of flooding on seeds of a number of species of forage crops. The crops included have proven to be of value on low lying spring flooded land or are under test to determine their possible usefulness. The results of this investigation are presented.

METHOD

The investigation was conducted in the greenhouse. Owing to limited space, it was necessary to run two separate experiments, each including seven different species of grasses and legumes. These were as follows:

Experiment I

<i>Trifolium hybridum</i> L.....	Alsike clover
<i>Melilotus alba</i> Desv.....	Sweet clover
<i>Medicago media</i> Pers.....	Alfalfa
<i>Phalaris arundinacea</i> L.....	Reed canary grass
<i>Bromus inermis</i> Leyss.....	Bromegrass
<i>Phleum pratense</i> L.....	Timothy
<i>Festuca elatior</i> L.....	Meadow fescue

¹ Contribution from the Forage Plants Division, Experimental Farms Service, Ottawa, Canada. Presented at the annual meeting of the American Society of Agronomy, Fort Collins, Colorado, August 24-27, 1948.

² Assistants in Forage Plants.

³ Heinrichs, D. H., and R. E. McKenzie. The effect of flooding on emergence of Reed canary grass seed. *Sci. Agr.* 27 : 4, 171-174, April, 1947.

Experiment II

<i>Trifolium pratense</i> L.....	Altaswede red clover
<i>Trifolium fragiferum</i> L.....	Strawberry clover
<i>Agropyron elongatum</i> (Host) Beauv....	Tall wheatgrass
<i>Agropyron intermedium</i> (Host) Beauv..	Intermediate wheatgrass
<i>Agropyron trachycaulum</i> (Link.) Malte var. <i>typicum</i> Fern.....	Slender wheatgrass
<i>Elymus virginicus</i> L. var. <i>submuticus</i> Hook.....	Virginia wild rye
<i>Alopecurus pratensis</i> L.....	Meadow foxtail

The first experiment started in January and concluded in April, 1947, while the second began in October, 1947 and terminated in January, 1948. The treatments in both experiments were identical. They consisted of four periods of flooding, 3, 6, 9 and 12 weeks, and a check. Germination tests were made on all seed lots prior to each experiment. One hundred seeds of each species were placed on top of two and one-half inches of dry loam soil in the bottom of gallon crocks. The seeds then were covered with an additional one-half inch of soil. Immediately after planting, all crocks excepting the checks were filled with water to a depth of six inches above the soil surface and maintained at this level until drained. The flooded crocks were drained at intervals of 3, 6, 9 and 12 weeks. The soil in the check crocks was merely kept moist enough to produce maximum emergence of the seeds. The position of the treatments was at random in each of the four replicates used, and the seven species were randomized in each treatment.

Following drainage the first emergence, if any, in each crock was noted. Subsequently, daily counts were made until such time as no further emergence occurred.

RESULTS AND DISCUSSION

The notes recorded from the two experiments showed that flooding delayed emergence as compared to the checks. Emergence in the check crocks began five to ten days after planting, but in the flooded crocks it did not begin until 16 to 20 days after draining. This delay, no doubt, was due to the water-logged condition of the soil which existed. As soon as the soil dried out sufficiently to become aerated, emergence began in those cases where flooding had not totally destroyed seed viability. By increasing the flooding period, no increase was noted in the length of time required for emergence after draining. Species which emerged after 12 weeks' flooding did not take any longer to do so than after three weeks' flooding.

The average per cent emergence of each species from each period of flooding is presented in Table 1. Per cent emergence was determined by—

$$\frac{\text{Number of seeds emerging}}{\text{Germination of the species}} \times 100$$

From an examination of the data in Table 1, it will be seen that flooding materially affected the emergence of several of the species included in the two experiments. As a group, the legume seeds were unable to endure

TABLE 1.—AVERAGE PER CENT EMERGENCE OF SEEDS FOLLOWING FLOODING

Species	Period of flooding				
	Check	3 Weeks	6 Weeks	9 Weeks	12 Weeks
<i>Experiment I—</i>					
Reed canary	75.0	96.4	87.1	90.3	91.1
Timothy	70.4	73.6	66.6	54.6	57.0
Bromegrass	85.5	70.0	50.9	52.1	25.7
Meadow fescue	91.9	66.6	49.1	31.7	16.9
Alsike clover	72.7	61.8	14.4	—	—
Alfalfa	71.4	53.3	—	—	—
Sweet clover	37.0	—	—	—	—
<i>Experiment II—</i>					
Slender wheatgrass	89.1	82.6	76.9	78.4	51.7
Virginia wild rye	77.7	48.6	57.1	47.8	38.5
Tall wheatgrass	92.2	57.8	41.2	28.9	27.1
Meadow foxtail	99.9	38.8	38.6	23.6	16.4
Intermediate wheatgrass	97.3	17.7	3.5	—	—
Strawberry clover	96.5	5.0	3.5	—	—
Altaswede red clover	89.2	2.8	—	—	—

even the shorter periods of flooding. Alsike clover and alfalfa were the two best species of those tested and both emerged fairly well after three weeks' flooding. No alfalfa emerged after six weeks' flooding, and the emergence of alsike was slight. Sweet clover, strawberry and altaswede red clover had only negligible emergence at the end of three weeks' flooding.

Seed of the grasses included in the two experiments was able to remain viable, in varying degrees, even after the longest periods of flooding. The exception among the nine grasses was intermediate wheatgrass, which had a low emergence after three weeks' flooding and practically none after six weeks. Meadow fescue and meadow foxtail both emerged at the end of the twelve-week flooding period, but their emergence was considerably reduced as compared to the checks. Meadow fescue held up better throughout than did meadow foxtail. Virginia wild rye, bromegrass and tall wheatgrass constitute a group whose seeds were able to endure rather well, even the longest flooding period. After twelve weeks' flooding, these species still emerged 38.5, 25.7 and 27.1 per cent, respectively. Virginia wild rye and bromegrass held up better throughout than tall wheatgrass, which dropped sharply in emergence following three weeks' flooding.

The outstanding species was Reed canary grass, whose seed was apparently unaffected by the length of the flooding period. Slender wheatgrass and timothy ranked next, both species emerging over 50 per cent after twelve weeks of flooding.

While the data from these two experiments indicate that seed of several forage species is able to endure long periods of flooding in the greenhouse, the practical significance of the results remains to be confirmed by field tests. Among the grasses tested, it so happens that the best six species in this respect, namely, Reed canary, slender wheatgrass, timothy, Virginia wild rye, bromegrass and tall wheatgrass, have either proven to be of considerable value for use on low lying spring-flooded lands, or are

showing promise in this respect. It is possible that late fall seeding of these species would be successful on areas that are flooded for fairly long periods in the spring.

It is unfortunate that none of the legumes tested was able to endure more than a short period of flooding as they are useful additions to forage mixtures. It would seem unlikely that higher emergence would occur in the field than in the greenhouse. However, it is possible that alsike clover and alfalfa could be seeded in mixtures in locations where the spring flood period was of relatively short duration.

SUMMARY

On spring flooded areas in Western Canada it is usually impossible to seed forage crops until June or early July. A preferable time to seed would be in the late fall just prior to freeze up, provided seed of the crops used was able to endure the spring flooding period and emerge enough thereafter to produce a satisfactory stand.

In greenhouse experiments, the relative ability of 14 species of forage crop seeds to remain viable through periods of flooding from 3 to 12 weeks was determined. Seed of the grasses was able to endure flooding better than the legumes tested. Reed canary grass seed (*Phalaris arundinacea* L.) was particularly outstanding, showing no decrease in emergence after 12 weeks of flooding. Next in order were slender wheatgrass (*Agropyron trachycaulum* (Link.) *Malte* var. *typicum* Fern.) and timothy (*Phleum pratense* L.), both emerging about equally well from all flooding periods. Seed of Virginia wild rye (*Elymus virginicus* L. var. *submuticus* Hook.), brome grass (*Bromus inermis* Leyss.) and tall wheatgrass (*Agropyron elongatum* (Host.) Beauv.) showed good ability to endure flooding as well but ranked below timothy and slender wheatgrass. Emergence of meadow fescue (*Festuca elatior* L.) and meadow foxtail (*Alopecurus pratensis* L.) was reduced considerably by twelve weeks' flooding. The only grass seed which failed to emerge to any extent after flooding was intermediate wheatgrass (*Agropyron intermedium* (Host.) Beauv.). Although a small percentage emergence was recorded following three weeks' flooding, there was practically none after six weeks.

Of the five legumes included in the investigation, strawberry clover (*Trifolium fragiferum* L.), alsaswede red clover (*Trifolium pratense* L.), sweet clover (*Melilotus alba* Desv.), alsike clover (*Trifolium hybridum* L.) and alfalfa (*Medicago media* Pers.), only seed of the latter two species was able to endure flooding but for considerably shorter periods than the grasses.

Subject to corroboration under field conditions, the results indicate several grass species might be successfully seeded in the late fall on areas flooded in the spring for long periods. Where the flooding was of short duration, alfalfa and alsike clover possibly could be included in mixtures.

A STUDY ON FIELD EXPERIMENTS OF SEMI-LATIN SQUARE DESIGN¹

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INTRODUCTION

In 1931, "Student" (11) announced a balanced arrangement which he called "equalized randomized block", and recommended to a horticulturist that he test 10 varieties or treatments in 5 replicates with double local control as in a common latin square. The same design, as discussed by Yates (13), was put forward independently by Pitman of Tasmania under the designation "semi-latin square". Snedecor (9), in 1934, also gave a similar pattern for the use of this design in testing 16 varieties arranging into a (4×4) latin square with 4 varieties in each latin square plot. This design was then used quite extensively by several workers in carrying on agricultural experiments. Published results of experiments arranged as semi-latin squares include work by Pope (7) in Arkansas, Stringfield, Lewis and Pfaff (10) in Ohio, Zuber and Robinson (14) in Iowa, Riddle and Baker (8) in California and Harrington (4) in Saskatchewan.

In "Student's" original plan, the assignment of ten varieties or treatments, A to J, to the plots in the first block was random, but each successive block had its arrangement more and more controlled, so that (1) each of the five columns contained one plot only of the ten varieties and (2) varieties A, D, E, F and J occurred in the top row of the blocks three times and in the lower row twice, while for B, C, G, H, I the position was reversed. This arrangement is similar to the "split-plot latin square" in general form excepting that in the latter, the same group of varieties is used for each of that latin square plots or main plots, while for the semi-latin square, there is no such restriction. The statistical treatment for these two designs is also different. For the split-plot latin square, the design provides two estimates of error for each experiment, one for the comparison of varieties falling in the same groups and the other for the comparison of varieties falling in different groups. The first standard error is derived directly from the sub-plot error. For the second, the mean of the main-plot and sub-plot errors are weighted in the ratio 1: k, where k is the number of varieties in each group. Since in the semi-latin square the varieties are not arranged in groups in the main plots, it is impossible to divide the analysis of variance into two parts as in the split-plot latin square. Thus the resultant estimate of error is different from that of the "split-plot latin square", and may have some bias as mentioned by Yates (12, 13).

In the present study the standard error distribution of 133 field experiments of the semi-latin square arrangement was analysed for the following purposes: (1) to estimate the relative efficiency of the design compared with the randomized block design; (2) to study the nature of the bias in the estimation of error; (3) to calculate the fractional bias with actual experimental data and to suggest methods of adjusting this bias.

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RELATIVE EFFICIENCY OF SINGLE AND DOUBLE RESTRICTIONS OF THE DESIGN

The Field Husbandry Department of the University of Saskatchewan used the semi-latin square design for varietal trials as early as 1933. During the fourteen years from 1933 to 1946, out of a total of 523 experimental designs used 133 were semi-latin squares. The crop distribution of these 133 experiments was: 44 on wheat, 45 on barley, 39 on oats, 3 on rye, 1 on flax and 1 on peas. The standard errors in per cent of these experiments were classified and are presented by crops in Table 1.

The average standard error for the 133 experiments was 14.8 per cent which was about the same size as the average standard error of 133 randomized block experiments (14.6 per cent) and much larger than that of 81 experiments of lattice designs (12.0 per cent), all of which were run by the same department and reported by Ma and Harrington (6) in 1948. The semi-latin square design is so arranged that it can be considered as a common randomized block test with either rows or columns taken as blocks and the data analysed as a randomized block test with only a single restriction. An attempt was made to ascertain the relative efficiency of this design and the common randomized block arrangement. The relative efficiency has been calculated in the same manner as Yates (12) used in studying the efficiency of the latin square compared with the randomized block. Since the ranges of the percentages of efficiency were large, with a maximum as high as 1443 per cent in one case, the median value was used to express the average percentage of efficiency. The results for the different crops are given in Table 2.

The efficiency of the semi-latin square compared with the randomized block design was much greater when columns were used as blocks than when rows were used as blocks. This was expected since the bulk of the semi-latin squares were about twice as long as wide. Thus the columns (which ran lengthwise of the design) accounted for less soil variability than the rows and were a less efficient local control than the rows in a large proportion of the semi-latin squares. The semi-latin square would be expected to show greater efficiency when compared with randomized blocks using the columns (with their relative low efficiency in local control), as blocks than when compared with randomized blocks using the rows (with their relative high efficiency in local control) as blocks.

The results showed the efficiency of the semi-latin square compared with the randomized block design to be: for wheat, 189 per cent with columns used as blocks and 122 per cent with rows used as blocks; for barley, 136 per cent and 116 per cent, respectively; for oats, 186 per cent and 109 per cent; for all three crops (133 experiments) 168 per cent and 118 per cent, respectively.

A study of Table 2 also reveals that when rows were used as blocks, in a randomized block plan, 2 experiments out of 44 in wheat, 10 experiments out of 45 in barley and 15 experiments out of 39 in oats, a total of 27 experiments of a semi-latin square design out of 133 were less efficient than the randomized block. When columns were used as blocks, only 1 experiment in wheat and 1 experiment in barley, or a total of 2 out of 133 were less efficient.

TABLE 1.—DISTRIBUTION OF STANDARD ERRORS IN PER CENT OF 133 EXPERIMENTS OF SEMI-LATIN SQUARE DESIGN

S.E. in per cent	Wheat	Barley	Oats	Rye	Flax	Peas	Total
4.1—8.0	3	6	4	—	—	—	13
8.1—12.0	12	10	14	—	—	—	36
12.1—16.0	13	11	7	2	—	1	34
16.1—20.0	10	9	8	1	1	—	29
20.1—24.0	2	3	4	—	—	—	9
24.1—28.0	2	3	1	—	—	—	6
Over 28.0	2	3	1	—	—	—	6
Total experiments	44	45	39	3	1	1	133
Average S.E.	14.6	15.3	14.0	16.4	20.0	15.7	14.8

FRACTIONAL BIAS OF THE ESTIMATED ERROR OF THE SEMI-LATIN SQUARE

Yates (12) in 1935, first criticized this design for its biased error. It was his opinion that it is only possible to make unbiased estimates of the appropriate errors when the treatments or varieties are grouped so to give the equivalent of a split-plot arrangement. Goulden (3) in reviewing the methods used for testing a large number of varieties referred to Yates' suggestion that this design suffers from a biased error and showed that the bias will usually be positive. Cox, Eckhardt and Cochran (1) and Zuber (14) also condemned the design in favour of the incomplete block designs. Riddle and Baker (8) considered that the experimental error estimates on semi-latin squares were not biased in the sense of Yates' criticism, although the method used by them for test is of doubtful validity.

As far as the writers known, there has been no detailed study made on the problems of the bias in the semi-latin square; yet such a study appears well worth-while. The problems may be enumerated as follows: (1) What direction does the bias take? Is it an under- or an over-estimation of the experimental error? (2) What is the size and what are the possible limits of the bias? (3) How may the bias be estimated from experimental data? (4) How may the bias be adjusted in actual field experimental results?

The bias in the semi-latin square arises because plots in the same group are correlated. The reason is the same as that for the main plot error usually being larger than the sub-plot error in the split-plot latin square. This can be illustrated as follows: Suppose that there are p k varieties, arranged in a $(p \times p)$ latin square with k plots or k varieties in each group. If σ^2 is the variance of an individual plot, and if plots in the same group have an intra-class correlation r , then it may be shown that the expected value of the true error mean square is equal to

$$T = \sigma^2 \left(1 - \frac{(k-1)}{(pk-1)} r \right) \quad (1)$$

On the other hand, the expected value of the error mean square that is obtained from the analysis of variance of the semi-latin square is equal to

$$E = \sigma^2 \left(1 - \frac{2(k-1)}{(pk-2)} r \right) \quad (2)$$

TABLE 2.—THE RELATIVE EFFICIENCY OF SEMI-LATIN SQUARE COMPARED TO COMMON RANDOMIZED BLOCK EXPERIMENTS

Percentage relative efficiency	Wheat		Barley		Oats		Rye		Flax		Peas		Total	
	Row*	Col.†	Row	Col.	Row	Col.	Row	Col.	Row	Col.	Row	Col.	Row	Col.
80-100	2	1	10	1	15	—	—	—	—	—	—	—	27	2
100-120	19	6	16	14	10	10	—	2	—	—	—	—	45	33
120-140	8	3	11	9	8	3	3	—	—	—	—	—	30	15
140-160	3	5	2	3	1	4	—	—	—	—	1	—	7	12
160-180	4	4	3	5	2	1	—	1	—	—	—	—	9	11
180-200	1	7	—	1	1	5	—	—	1	—	—	—	3	13
200-220	2	5	3	3	1	3	—	—	—	—	—	—	6	11
220-240	—	1	—	1	—	—	—	—	—	—	—	—	—	2
240-260	3	2	—	2	1	1	—	—	—	—	—	—	4	5
260-280	1	2	—	1	—	1	—	—	—	—	—	—	1	4
280-300	1	—	—	—	—	—	—	—	—	—	—	—	1	—
Over 300	—	8	—	5	—	11	—	—	—	—	—	1	—	25
Total number of experiments	44	44	45	45	39	39	3	3	1	1	1	1	133	133
Median percentage	122.5	188.6	115.6	136.4	109.0	186.0							117.6	168.2
Ranges of percentage	98.3-284.5		93.6-207.9		88.2-258.8								88.2-284.5	
Ranges of percentage		93.7-994.2		100.0-530.5		101.0-1443.1							93.6-1443.1	

* The rows are used as blocks.

† The columns are used as blocks.

T is different from E , and this difference will indicate the bias in the analysis of variance error. By simplification, the fractional bias can be calculated as formula (3)

$$b = \frac{(E - T)}{T} = \frac{-pk(k-1)r}{(pk-2)[(pk-1) - (k-1)r]} \quad (3)$$

Since r must lie between $+1$ and $-\frac{1}{(k-1)}$, so the upper and lower limits for the fractional bias are given as:

$$\text{Lower limit (when } r = +1) = \frac{-p(k-1)}{(p-1)(pk-2)},$$

$$\text{Upper limit (when } r = -\frac{1}{(k-1)}) = \frac{1}{(pk-2)}$$

Under practical field conditions, r usually will be positive, so that the design gives an under-estimate of the true error, i.e. a negative fractional bias is usually obtained.

In practice, if one wishes to find out the extent of bias of a semi-latin square experiment, he should calculate or estimate from the experimental data the intra-class correlation between the plots of a group. This cannot be done from the actual semi-latin square experiments, because the varieties are different in each latin square plot, and the plot differences in one group are confounded with the varietal differences. But r can be estimated either from uniformity data, or from the results of split-plot latin square experiments. Suppose there is a split-plot latin square for testing pk varieties arranged into $(p \times p)$ latin square with k varieties per group. Then the expected values of main-plot and sub-plot errors are given as follows:

$$\text{Main-plot error } E_a = \sigma^2 (1 + (k-1)r) \quad (4)$$

$$\text{Sub-plot error } E_b = \sigma^2 (1 - r) \quad (5)$$

Therefore the intra-class correlation coefficient r can be estimated from the main and sub-plot errors actually calculated from the experimental results i.e.

$$r = \frac{E_a - E_b}{E_a + (k-1)E_b} \quad (6)$$

and

$$b = \frac{-p(k-1)(E_a - E_b)}{(pk-2)[(p-1)E_a + p(k-1)E_b]} \quad (7)$$

Instead of estimating r and b from the difference of E_a and E_b , we may simplify the calculation by using the ratio of E_a to E_b .

Let

$$a = \frac{E_a}{E_b}, r = \frac{a-1}{a+(k-1)} \quad (8)$$

and

$$b = \frac{-p(k-1)(a-1)}{(pk-2)[(p-1)a + p(k-1)]} \quad (9)$$

APPLICATION OF FRACTIONAL BIAS FORMULA TO ACTUAL EXPERIMENTAL RESULTS

Seventeen experiments of split-plot latin square design were conducted at the University of Saskatchewan in 1936 and 1937 on wheat, barley and oats as described by Ma and Harrington (6). The number of varieties tested varied from 8 to 48. Eleven experiments were of the (4×4) type and the remaining were (6×6) . It is interesting to apply the formula given above to the data of these 17 experiments in order to estimate their intra-class correlation coefficients and the fractional bias. The significance of the coefficients may be tested by means of the transformation of r to

z , where $z = \frac{1}{2} \log_e \left(\frac{1 + (k-1)r}{1-r} \right)$ with the standard error of z equal to

$\sqrt{\frac{k}{2(k-1)(n-2)}}$ with n = total number of groups and k = number of

plots per group. Or the significance may be tested easily by the variance ratio of E_a to E_b i.e. the F -value with degrees of freedom $(p-1)(p-2)$ and $p(p-1)(k-1)$, respectively. The results are given in Table 3.

Only one of the 17 experiments showed a negative correlation; in other words, the error calculated for that particular semi-latin square would be an over-estimation of the real error. But this over-estimation was so small, only 0.3 per cent, that it had no significance. On the other hand, the other 16 experiments showed positive correlations with sizes ranging from $+0.027$ to $+0.828$. The underestimation of error mean square ranged from 0.7 per cent to 22.3 per cent of the real error mean squares. The test of significance of these 17 coefficients by transformed z values showed 13 to be significant and for these demonstrated a real correlation between plots within the same group.

TABLE 3.—THE CORRELATION COEFFICIENTS AND FRACTIONAL BIAS PERCENTAGES OF SEVENTEEN SPLIT-PLOT LATIN SQUARE EXPERIMENTS

Crops	p	k	Number of varieties	r coefficient	Z \pm S. E.	$\frac{(E - T)^b}{T} \times 100$
Barley	4	4	16	0.6016	0.9758 \pm 0.2182*	-15.6
Wheat	4	8	32	0.7001	1.4903 \pm 0.2020*	-20.0
Wheat	4	7	28	0.4197	0.9009 \pm 0.2042*	-11.1
Wheat	4	7	28	0.7718	1.6034 \pm 0.2042*	-22.3
Wheat	4	12	48	0.0270	0.1426 \pm 0.1975	- 0.7
Wheat	4	7	28	-0.0116	-0.0417 \pm 0.2042	+ 0.3
Wheat	4	6	24	0.1975	0.4544 \pm 0.2069*	- 4.9
Wheat	4	12	48	0.2258	0.7521 \pm 0.1975*	- 5.8
Wheat	4	10	40	0.3716	0.9665 \pm 0.1975*	- 9.9
Oats	4	2	8	0.3965	0.4186 \pm 0.2672	- 8.0
Barley	4	2	8	0.1407	0.1426 \pm 0.2672	- 2.7
Wheat	6	3	18	0.2728	0.3757 \pm 0.1483*	- 3.7
Wheat	6	4	24	0.8283	1.5053 \pm 0.1400*	-13.2
Wheat	6	3	18	0.4403	0.6059 \pm 0.1483*	- 6.1
Wheat	6	3	18	0.6909	1.0206 \pm 0.1483*	-10.0
Wheat	6	3	18	0.6227	0.8917 \pm 0.1483*	- 8.9
Wheat	6	3	18	0.6150	0.8781 \pm 0.1483*	- 8.8

* = Significant.

Since the semi-latin square design usually underestimates the real error mean square, a method of adjusting for this negative bias is necessary. But this adjustment is only possible when the intra-class correlation between plots is obtained previously. One method of achieving this adjustment is to calculate the extent and the average value of the correlation coefficient based on accumulated actual field experimental data of split-plot latin square designs. Another method is to estimate the r value from data from uniformity trials run on land to be used for conducting the semi-latin square experiments.

Considering the first method, the average correlation coefficient of the 17 experiments of split-plot latin square design was estimated by Fisher's (2) transformed z -method. First, the average of the 17 values was obtained by weighing each z by its reciprocal of corresponding variance. Then the products were summed up and divided by the sum of weights to give the average z value, which was 0.8216. This was transformed back to r , giving $+0.4247$.

To illustrate the adjustment of a semi-latin square error where the intra-class correlation is known, we can assume a (4×4) type for testing 24 varieties with 6 plots per group. Let us also assume that the mean and variance of such an experiment are 120 and 144, respectively, and $r = +0.4247$. The fractional bias is then determined as

$$b = \frac{-4 \times 6 (6 - 1) \times 0.4247}{(4 \times 6 - 2) [(4 \times 6 - 1) - (6 - 1) \times 0.4247]} = -.111 \text{ or } -11.1\%$$

The range of r of this design is from -0.2 to $+1$ thus the corresponding possible limits of the fractional biases range from $+4.5$ per cent to as high as -30.3 per cent.

The adjusted variance is calculated as follows:

$$E^1 = \frac{E}{(1 + b)} = \frac{144}{(1 - 0.111)} = 161.98 \quad (10)$$

Where E^1 = adjusted variance and E = actual variance.

The unadjusted standard error is 12 and equal to 10 per cent of the mean, while the adjusted standard error is 12.72 or 10.6 per cent of mean.

The second method is the use of uniformity trials. Uniformity trials conducted in the field before running experiments give an idea of the association of the plots within groups and this correlation of plots reveals the heterogeneity of the soil and its suitability for experimentation. Suppose pk varieties with $(p \times p)$ latin squares and k -plots per main plot are superimposed on a uniformity trial. The partition of degrees of freedom for such an experiment is $(p - 1)$ for rows, $(p - 1)$ for columns, $(p - 1)^2$ for error a or (E_a) , $p^2 (k - 1)$ for error b or (E_b) and $(p^2 k - 1)$ for the total. A correlation coefficient is then estimated from error (a) and error (b). Such a correlation coefficient was used by Harris in 1915 and 1920 as given in Hayes and Immer (5) for studying soil heterogeneity; he termed it the coefficient of soil heterogeneity. Harris used uniformity data obtained from several investigators and then measured, in terms of intra-class correlation, the extent to which contiguous plots resembled each other. The larger the coefficient the greater was the heterogeneity. He obtained quite significant coefficients for various crops.

TABLE 4.—VARIANCES AND CORRELATION COEFFICIENTS OF FOUR TRIALS OF LATIN SQUARES SUPERIMPOSED ON UNIFORMITY TRIALS DATA

	D.F.	First trial variances	Second trial variances	Third trial variances	Fourth trial variances
Rows	2	27.35	34.77	4.10	50.17
Columns	2	537.52	991.60	10.18	0.44
Error (a)	4	75.35	42.78	14.54	3.16
Total	8	178.90	277.98	10.84	14.37
Error (b)	27	27.92	23.90	12.15	5.95
Total	35	62.43	81.98	11.85	7.87
r		0.297	0.165	0.048	-0.133
F		2.69	1.79	1.20	1.88

Significant F for $n_1 = 4$, $n_2 = 27$. 5% F = 2.73.

Four samples of (3×3) latin squares with 4-plots per group for testing 12 varieties were superimposed on the potato uniformity trials reported by Kalamker and discussed by Yates (13). The correlation coefficients of these four trials are given in Table 4. None of the correlation coefficients was significant at the 5 per cent point. In this example the soil was shown to be quite homogeneous and the bias in a semi-latin square experiment conducted on such land would not be appreciable.

DISCUSSION

The main criticism of the semi-latin square design as stated by many workers is the bias in the estimated error. The detailed study of this design revealed that bias arises from the correlation of the plots within the latin square plot or main plot. This intra-class correlation may be shown to be significant or non-significant depending upon the heterogeneity of the soil in which the experiments are conducted. Also this correlation may be either positive or negative, that is, the bias may be sometimes in one direction and sometimes in the other. In most cases the real error is underestimated.

Where it is desired to use this design in field test of varieties, the bias should be adjusted by first estimating the correlation coefficient either from the previous data of the split-plot latin squares or from uniformity trials, and then applying the formulae as given above. With either method the adjustment is completely dependent upon the accuracy of the correlation value secured. The actual results of 17 split-plot latin square experiments, as given in Table 3, show a wide variation of the obtained correlation coefficients, the values ranging from -0.01 to $+0.83$. So it is of doubtful value to make use of such an average correlation coefficient secured from previous uniformity trials or split-plot experiments for adjusting the subsequent experiments. Moreover, the writers feel that a wide range of correlation coefficients may be expected under variable soil-climatic conditions such as obtain in Central Saskatchewan. Furthermore,

even if an accurate correlation coefficient is obtainable for adjustment, the land and labour devoted to carrying out the uniformity trial or split-plot latin square experiments would also reduce the efficiency of the semi-latin square design. The bias may be left out of consideration if fairly homogeneous land is used for a test. However, if the soil is uniform the argument for using a semi-latin square loses its value.

As compared to the randomized block arrangement, the semi-latin square design allows the varieties tested to be distributed more evenly throughout the field. Also, by having each variety appear only once in each row and once in each column, more variation due to soil heterogeneity may be eliminated from the estimated error in the analysis of variance. This is demonstrated very clearly in the present study of the efficiency of the semi-latin square design compared to that of the randomized block for 133 actual field experiments. However, the writers (6) have shown that when the number of varieties is not very large, say under 10, the semi-latin square design, regardless of bias, may be considered less satisfactory than the latin square, and for tests of a larger number of varieties, is distinctly inferior to the lattices.

The importance of the comparison of the efficiency of the semi-latin square with that of the lattice designs appears to warrant a somewhat detailed discussion here. In the earlier paper of the writers (6) they reported on the investigation of the distribution of the percentage standard errors of 523 field experiments of different designs conducted by the Field Husbandry Department of the University of Saskatchewan, and found the distribution of the average standard error in per cent of the semi-latin square and of the lattice according to the number of varieties used was as follows:

Number of varieties per test	Below 11	11-20	21-40	Above 40
Semi-latin square	10.98 (10)*	14.15 (51)	15.48 (64)	17.93 (8)
Lattice	—	10.36 (10)	12.12 (53)	12.60 (18)

* The number within the bracket indicates the number of tests.

For the semi-latin square, the average standard error in per cent increased rapidly when the number of varieties per test increased. The average standard error of each class for number of varieties was larger for the semi-latin square than for the lattice in every corresponding class and the difference was largest when the number of varieties per test was above 40. The efficiency of the semi-latin square compared with the lattice was calculated for each class. For the class with 11-20 varieties, the semi-latin square was only 54 per cent as efficient as the lattice; for the 21-40 class the relative efficiency was 61 per cent and for the above 40 class the semi-latin square was only 49 per cent as efficient as the lattice. While these results do not show a trend toward a reduction in the efficiency of the semi-latin square with increased number of varieties, it is well known that such

a trend may be expected. Even without considering the biased estimate of the experimental error, the semi-latin square is shown by these results to be far inferior to the lattices for testing a moderate or large number of varieties.

SUMMARY AND CONCLUSIONS

1. The data from 133 field experiments designed as semi-latin square and conducted by the Field Husbandry Department of the University of Saskatchewan during the years 1933 to 1946 were used to study the efficiency of the semi-latin square in relation to the common randomized block design.

2. It was found that when rows were taken as blocks, to simulate a randomized block design, the semi-latin square was 18 per cent more efficient than the randomized block. When columns were used as blocks the semi-latin square was 68 per cent more efficient than the randomized block.

3. The semi-latin square design shows a bias in the estimated error, the bias arising from plots in the same main plot or group being correlated. Formulae for estimating this bias are derived with possible lower and upper limits. The bias may be positive or negative and may be significant or non-significant depending upon the soil heterogeneity.

4. The intra-class correlation coefficient of the plots of the same group can be estimated from uniformity data or from the experimental results of the split-plot latin square but cannot be estimated from actual semi-latin square experiments. Seventeen field experiments designed as split-plot latin squares were used to estimate this correlation. Excepting for one experiment which showed a non-significant negative correlation, the other sixteen showed coefficients varying from + 0.03 to + 0.83. Thirteen out of 16 of these positive coefficients proved significant. The underestimation of the error mean squares ranged from 0.7 per cent to 22.3 per cent. The average correlation coefficient was calculated by means of transformed Z-values and was found to be + 0.4247.

5. Four samples of (3×3) latin square for testing 12 varieties were superimposed on potato uniformity data. The correlation coefficients found were + 0.297, + 0.165, + 0.048 and - 0.133, respectively. None appeared to be significant by the F-test, which indicated that the bias may not be important in reasonably uniform soil.

6. The semi latin square design has but little practical use for testing a large number of varieties not because of its biased estimate of experimental error, which might be adjusted, but because of its low efficiency in accuracy as compared to the lattice design.

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BOOK REVIEW

PRACTICAL PLANT ANATOMY, by A. S. Foster. 2nd Edition. D. Van Nostrand Co. (Canada) Ltd., 228 Bloor Street West, Toronto, Canada. 224 pages. 1949. \$3.25.

This compact volume is completely rewritten and extended to include the advances made in comparative and developmental anatomy since the first edition was published in 1942. Intended for use in the laboratory, it combines the functions of a source book and a manual of laboratory directions. With a minimum of discussion, the author attempts to guide the student in his reading and laboratory study. The organization of chapters follows the pattern: the cell, cell types, tissues, tissue systems, and organs. For each topic an introduction provides descriptions, a concise historical review, and a summary of the present status of knowledge of the subject. The laboratory directions, which follow the introduction, are divided into "Materials for Study" and "Suggested Drawings and Notes". The use of living material is encouraged. Descriptive materials are combined with suggestions directing attention to features that the student is expected to work out. Diagrams and notes are recommended in partial substitution for tedious drawings of complex tissues. Each chapter concludes with a list of references. Fundamentals are stressed throughout with less attention to variations in given cells and tissues. While the absence of all illustrations detracts from the usefulness of this text as a source book, the plan should promote independent study on the part of the student.

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